Emirates Journal of Food and Agriculture. 2021. 33(7): 589-599
doi: 10.9755/ejfa.2021.v33.i7.2736
http://www.ejfa.me/

RESEARCH ARTICLE

Genetic diversity analysis of Linseed (*Linum usitatissimum* L.) accessions using RAPD Markers

Beema Nagabhushanam, Mohammad Imran Mir, M. Nagaraju, E. Sujatha, B. Rama Devi, B. Kiran Kumar*

Molecular Genetics and Biotechnology Laboratory, Department of Botany, Osmania University, Hyderabad, Telangana, India

ABSTRACT

It is important to analyse the degree of genetic variation existing within the genome to extend the genetic base of linseed/flaxseed accessions in order to preserve, evaluate and use genetic resources accurately and successfully. The main aim of the current investigation was to evaluate the scope and spread of genomic variation across different linseed accessions by employing molecular markers (RAPD). The genomic DNA of 12 linseed accessions was amplified with 16 decamer RAPD primers that generated 81 total bands, among which 75 bands were polymorphic and 6 bands were monomorphic. Polymorphic band numbers varied from least 2 (OPS-11) to highest 10 (OPS-07). The magnitude of polymorphism ranged from 75% to 100% among all accessions with a mean of 93.15 % across all the accessions. The value of Polymorphic Information Content (PIC) varied from 0.133% to 0.708% with a mean of 0.45% for each primer. The maximum PIC value (0.708) was found with the primer OPS-07 and (0.702) with OPM-13. The primer OPS-03 showed the minimum PIC value (0.133). Two main different clusters -I and -II were seen in the cluster analysis depending on RAPD data. Cluster-II comprises one accession (IC 564585) that was the highly varied accession, whereas Cluster-I comprises of some sub clusters with all the remaining accessions. The Jaccard's similarity coefficient varied from 8.2 to 96.3%. The accessions BHU-A and BHU-B had the highest genetic similarity (96.3%), followed by BHU-B and IC 564605 (96.2%). More divergent accessions were discovered to be IC 564585, IC 564616, IC 564631, IC 564622, and IC 564630. The current investigation provides innovative knowledge to breeders on the germplasm of linseed that would be employed in subsequent research to improve linseed genotypes.

Keywords: Genetic diversity; *Linum usitatissimum*; Polymorphism; RAPD Markers

INTRODUCTION

Linseed (*Linum usitatssimum* L.), also known as flax seed, is an annual herb, self- pollinating, (Ragupathy et al., 2011; Yadava et al., 2012) diploid (2n=30) plant species from the Linaceae family. The plant grows up to 1.2 m in length with a delicate stem, leaves subsessile, 1-4 cm long, linear to linear-lanceolatea, attenuate at both ends, glabrous, 3-nerved. Linseed/flax seed grown for two purposes, its fiber and its seed oil, the fiber obtain from the stems is woven in to linen fabrics for uses in the home and in the industries and for clothing (Zohary, 1999). These plants are raised in Canada, India, United Kingdom, Ethiopia and USA Primarily for its fibre, oil and medicinal compounds. India holds the second position in the world in terms of linseed cultivated land after Canada while fourth in terms of production following Canada, China, and the United States of America (Yadava et al., 2012; Chandravathi et al., 2014). Unique health benefits of linseeds are Omega-3 fatty acid, high-lignan content, and mucilage gums (Bjelkova et al., 2012; E1-Beltagi et al., 2007; Schmidt et al., 2012; Hosseinian et al., 2004; Westcott and Muir, 2003), as a result it was found to be valuable product in the food sector.

Diversity is a crucial aspect of the effective programme of breeding. Higher germplasm diversity gives the breeders more choices when it comes to identify parents for improving necessities-based crop varieties. Initially, genetic variation analyses relied on morphological and biochemical markers such as isozymes (Mansby et al., 2000; Diederichsen et al., 2006; Saeidi, 2012), however, phenotypic traits are not only delicate to environmental conditions but they also labour-exhastive and time adsorbing. Presently several molecular markers are being employed for analysing genetic variation of crop plants among them are RAPD, AFLPs, and ISSRs (Patzk, 2001), since they do not need detailed genomic knowledge and are easier, least expensive and least labour-intensive than other DNA marker methods.

***Corresponding author:**
B. Kiran Kumar, Molecular Genetics and Biotechnology Laboratory, Department of Botany, Osmania University, Hyderabad, Telangana, India.
E-mail: kiran.nrcpb@gmail.com

Received: 01 April 2021; **Accepted:** 30 June 2021

RAPD markers have been successfully used in molecular diversity investigations of crops (Williams et al., 1990; Fu et al., 2003; Umesh et al., 2013; Diederichen et al., 2006). The reproducibility issue with RAPD can be overcome if factors such as DNA quantity and experimental conditions are carefully maintained across different sets off reactions (Ulloa et al., 2003) RAPD, ISSR and IRAP Markers were used to analyse the genetic diversity of flax accessions (Ziaravoska et al., 2012; Kumar et al., 2018; Srivastav et al., 2013). The objective of this investigation is to classify and assess the degree of genetic diversity within 12 accessions of linseed genotypes.

MATERIAL AND METHODS

Collection of plant material

Twelve accessions of linseed (*Linum usitatissimum* L.) were used for genetic diversity analysis and polymorphism information in this investigation. Of the 12 accessions, 10 accessions of linseed were collected from NPBGR, New Delhi, and two accessions from Banaras Hindu University, UP. The details of these accessions were presented in (Table 1). All the accessions were raised in green house conditions at University College of Science, Department of Botany, Osmania University, Hyderabad. After proper initial growth the tender young leaves were harvested from 12 different accessions of linseed, rinsed in double distilled water and covered in aluminium paper and stored at -20°C till the DNA extraction.

DNA extraction

The CTAB (Cetyltrimethyl ammoniumbromide) procedure (Doyle and Doyle, 1987) was used to isolate genomic DNA. Young leaves were pulverised to a fine powder in liquid nitrogen and mixed with 20 mL DNA extraction buffer (2% CTAB, 1.5M Nacl; 20 mM EDTA, 100 mM Tris-Cl, pH 8.0; 0.2% β-mercaptoethanal). After through mixing 20 µl of RNAase was mixed to it and incubated 37°C for 30 min. Equal amount of phenol: chloroform:isoamyl alchol (25:24:1) was added and centrifuged at 15,000 rpm for 15min. The DNA was precipitated in 100% ethanol (chilled), the DNA pellet was washed with 70% ethanol, dried in vacuum and dissolved in TE buffer (pH 8.0). Concentration of DNA samples were carried out using NanoDrop-1000 3.3.1 spectrophotometer (Thermo Scientific) and purity was determined by taking ultraviolet absorbance ratio at 260/280 on spectrophotometer and running 50 ng DNA sample on 1% agarose gel along with 1 Kb DNA ladder (Fig. 1).

Table 1: List of linseed accessions utilized in the current investigation

S.No.	Accessions no	Source of Linseed germplasm
1	IC 564616	NBPGR, New Delhi.
2	IC 564622	NBPGR, New Delhi.
3	IC 564631	NBPGR, New Delhi.
4	IC 564692	NBPGR, New Delhi.
5	IC 564630	NBPGR, New Delhi.
6	IC 564660	NBPGR, New Delhi.
7	IC 564685	NBPGR, New Delhi.
8	IC 564676	NBPGR, New Delhi.
9	IC 564624	NBPGR, New Delhi.
10	IC 564605	NBPGR, New Delhi.
11	BHU-A	Banaras Hindu, University, Varanasi, Uttar Pradesh
12	BHU-B	Banaras Hindu, University, Varanasi, Uttar Pradesh

PCR conditions

Sixteen decamer random primers (Table 2) were used for Polymerase chain reaction (PCR). Polymerase chain reaction was performed in an amount of 20 µl with 4 µl of 30 ng DNA template, 2.0 µl of 15pmol primers (Eurofins Genomics, Bangalore, India) 0.3 µl of 1.5U Taq polymerase (Genei, Bangalore), 2µl of 10x reaction buffer, 2 µl of 5 mM dNTPs (Genei, Bangalore) and 7.7µl of miliQ water. The amplification reactions were accomplished in a Master Thermo Cycler (Eppendorf), with an initial denaturation at 94°C for 5minutes, denaturation at 94°C for 1 minute, annealing at 35°C for 1 minute, extension at 72°C for 2 minutes and the final extension at 72°C 10 minutes for 40 cycles. The amplified fragments were electrophoresed on 2% agarose gel in 1x TAE buffer, pH 8.0 and were run in 100V for an hour range of every fragment was determined employing 1Kb DNA ladder (Genei, Bangalore, India). Gel documentation system (Kodak EDAS 290) was used to visualize the gel after it was stained with ethidium bromide and exposed to UV light.

RAPD data analysis

Every primer's definite and clearly visible amplified RAPD strands were carefully scored and encoded into a binary matrix, by 1 stand for the presence of a band and 0 stand for the absence of a band, amplified DNA fragments having equal migration were considered as similar bands and uncertain bands which could not be surely differentiated were not scored, DNA ladder was used to compare the position of amplified PCR bands and the binomial results obtained was utilized to evaluate polymorphism levels.

Pair-wise genetic similarity matrices of 12 linseed accessions were produced by Jaccard's co efficient of similarity (Jaccard, 1908) by using NTSYS-pc.2.02 software

Table 2: Amplification of 12 linseed accessions with 16 RAPD primers

S.no.	Primer	Sequence	TM (°C)	GC%	TB	No.of Bands	Mono	Mono (%)	PB	Poly (%)	Band Size (bp)	PIC
1	OPG-2	GGCACTGAGG	33.0	70.0	37	6	0	0	6	100	400-1500	0.619
2	OPG-5	CTGAGACGGA	28.9	60.0	63	8	2	25	6	75	200-2000	0.461
3	OPI-02	GGAGGAGAGG	33.0	70.0	NO AMPIFICATION/ NO PROPER AMPLIFICATION							
4	OPM-13	GGTGGTCAAG	28.9	60.0	36	7	0	0	7	100	300-2000	0.702
5	OPM-10	TCTGGCGCAC	33.0	70.0	79	8	1	12.5	7	87.5	500-3000	0.244
6	OPO-03	CTGTTGCTAC	24.8	50.0	44	7	0	0	7	100	200-3000	0.601
7	OPO-07	CAGCACTGAC	28.9	60.0	NO AMPIFICATION/ NO PROPER AMPLIFICATION							
8	OPS-03	CAGAGGTCCC	33.0	70.0	67	6	1	16.7	5	83.3	500-2000	0.133
9	OPO-12	CAGTGCTGTG	28.9	60.0	45	5	0	0	5	100	500-3000	0.326
10	OPS-11	AGTCGGGTGG	33.0	70.0	12	2	0	0	2	100	200- 800	0.576
11	OPS-07	TCCGATGCTG	28.9	60.0	53	10	0	0	10	100	500-1500	0.708
12	OPS-12	CTGGGTGAGT	28.9	60.0	36	4	0	0	4	100	600-1000	0.378
13	OPZ-01	TCTGTGCCAC	28.9	60.0	28	4	1	25	3	75	500-2000	0.510
14	OPZ-05	TCCCATGCTG	28.9	60.0	23	3	0	0	3	100	400-1500	0.438
15	OPZ-03	CAGCACCGCA	33.0	70.0	54	5	0	0	5	100	500-3000	0.189
16	OPU-01	CAGCACCGCA	28.9	60.0	40	6	1	16.7	5	83.3	500-1500	0.532
Total					617	81	6		75			
Mean					44.07	5.7		6.85		93.15		0.45

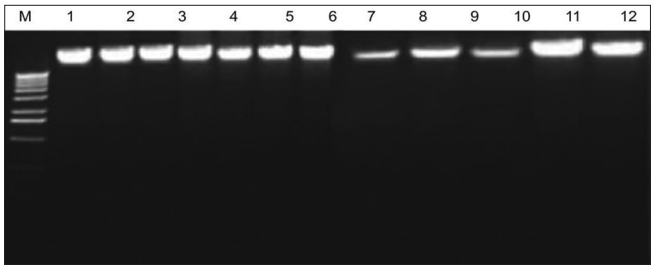

Fig 1. Agarose gel electrophoresis of Isolated DNA sample of Linseed accessions. Lane M =DNA lader. Lane:1= IC 564616, Lane:2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

programme (Rohlf, 2000). The dendrogram depending on the similarity matrix was developed by using Unweighted pair group method with arithmetic average (UPGMA) to assess the genetic relationship among the 12 accessions. Polymorphic information content (PIC) was calculated on the basis of (Roldan-Ruiz et al., 2000), formula i.e. PICi = 2fi(1-fi). Where PICi is the polymorphism information content of marker 1, fi is the frequency of the marker bands present and (1-fi) is the frequency of the marker bands absent.

RESULTS

In the current investigation, 12 linseed accessions (*Linumusitatissimum* L.) were characterised using RAPD analysis to establish their genetic correlation among themselves. To evaluate the 12 accessions, a total of 16 random primers were initially utilized, out of which 14 random primers produced distinct bands while 2 primers OPI-02 and OPI-07 did not result in any amplification. 14 primers produced 617 scorable bands and 81 total bands among which 75 polymorphic bands representing for an average of 93.15% polymorphism and 6 monomorphic bands represented an average of 6.85% monomorphism. The number of amplified bands varied from 2 to 10 with a molecular size ranging from 250 bp to 3000 bp for different primer used (Figs. 3-19). The maximum number of amplified bands (10) was produced from primer OPS-07 whereas the primer OPS-11 produced minimum number of amplified bands (2). The highest level of polymorphism (100%) was produced by OPG-2, OPM-13, OPO-03, OPO-12, OPS-07, OPS-11, OPS-12, OPZ-05 and OPZ-03 primers (Table 2).

Polymorphic information content (PIC)

The PIC values of twelve linseed accessions varied from 0.133 to 0.708 (Fig. 2). The maximum PIC value was recorded with RAPD random primer OPS-07 was (0.708) followed by (0.702) with RAPD random primer OPM-13 and (0.619) with RAPD random primer OPG-2. The lowest PIC value (0.133) was recorded with RAPD random primer OPS-03. PIC values were used to calculate differentiation capacities for each accession.

Cluster analysis and genetic similarity matrix

The RAPD markers results were statistically interpreted with the NTSYSpc.2.02e programme, and a dendrogram was developed depending on the similarity matrix using Unweighted Pair Group Method with Arithmetic Average (UPGMA) which clearly distinguished all of linseed accessions. A genetic correlation among twelve accessions was ranged from 8.2 to 96.3%. While least

similarity (8.2%) was obtained between accessions IC 564585 and IC 564676 it indicates these two accessions are considerably different and the highest similarity of 96.3% was observed between accessions BHU-A and BHU-B which indicates that these accessions are deeply associated with one another followed by BHU-B and IC 564605 96.2% (Table 3). The accession IC 564585 contained the least range of the pairwise genetic similarity coefficient, with all remaining accessions varying from 8.2 to 12.6. The accessions IC564585, IC564616, IC564631, IC564622, IC564512, and IC 564630 were identified to be the most divergent of all other accessions analysed in terms of genetic distance. The cluster analysis was done using jaccard's similarity coefficient to study the genetic diversity among these 12 linseed accessions. As shown in Figs. 20-22, the generated dendrogram was classified into 2 major cluster (I and II), there is only one accession (IC 564585) in the first cluster I and second cluster II comprises of 11 accessions, which were classified as two sub-clusters (IIA & IIB) the first sub cluster IIA comprises of 4 accessions and was split again into two (IIA_1 & IIA_2). The IIA_1 consists of two accessions IC 564616 and IC 564631, IIA_2 consists of two accessions namely IC 564622 and IC 564592. The second sub-cluster IIB consists of 7 accessions and was divided again into two sub- cluster (II B1& IIB2) sub-cluster IIB1 consists of only one accession IC 564660 and sub-cluster IIB2 consists of 6 accessions. Additionally, sub-cluster IIB2 classified into two sub-cluster (IIB2a& IIB2b), IIB2a consists with 2 accessions namely IC 564630 and IC 564624, IIB2b consists of 4 accessions namely IC 564676, IC 564605, BHU-A and BHU-B.

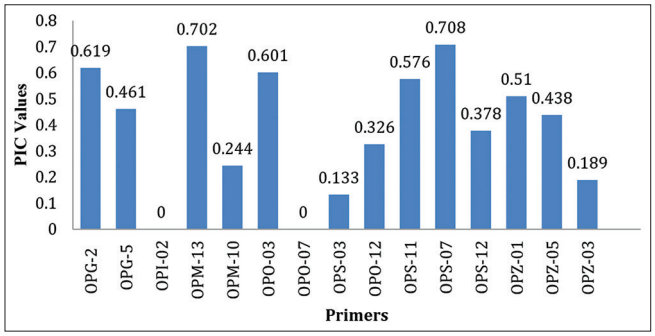

Fig 2. PIC values with 16 RAPD primers across the 12 linseed accessions.

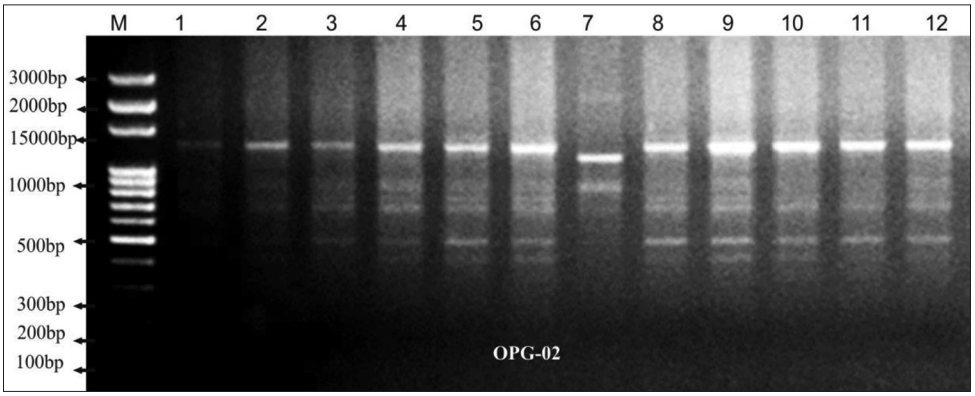

Fig 3. RAPD-PCR analysis of 12 linseed accessions with OPG-2 Random primer. Lane M =DNA lader. Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

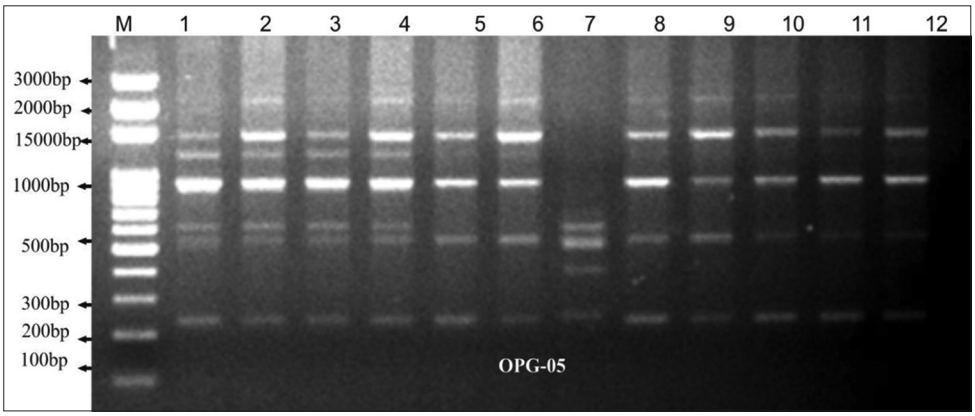

Fig 4. RAPD-PCR analysis of 12 linseed accessions with OPG-05 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

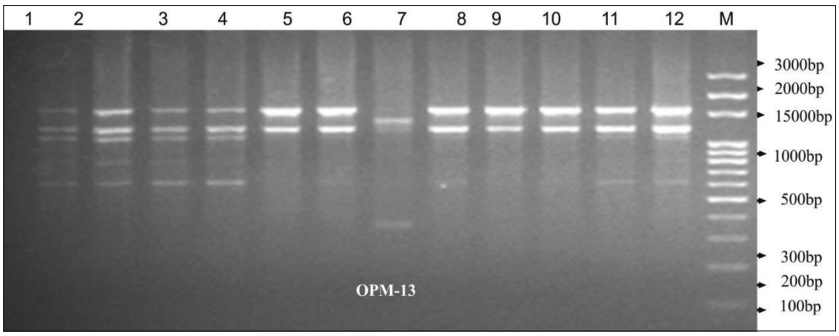

Fig 5. RAPD-PCR analysis of 12 linseed accessions with OPM-13 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

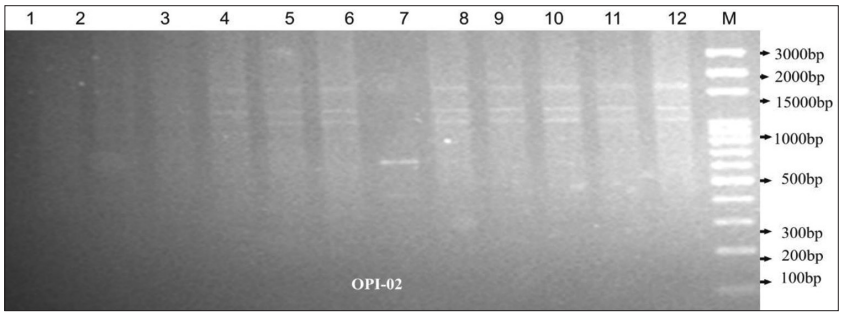

Fig 6. RAPD-PCR analysis of 12 linseed accessions with OPI-02 Random primer. Lane M =DNA lader. Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

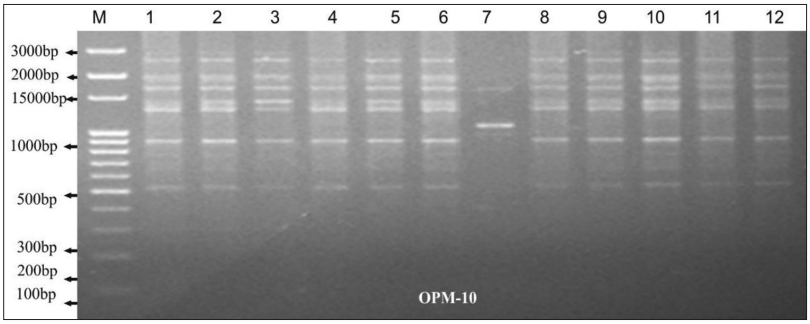

Fig 7. RAPD-PCR analysis of 12 linseed accessions with OPG-2 Random primer. Lane M =DNA lader. Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

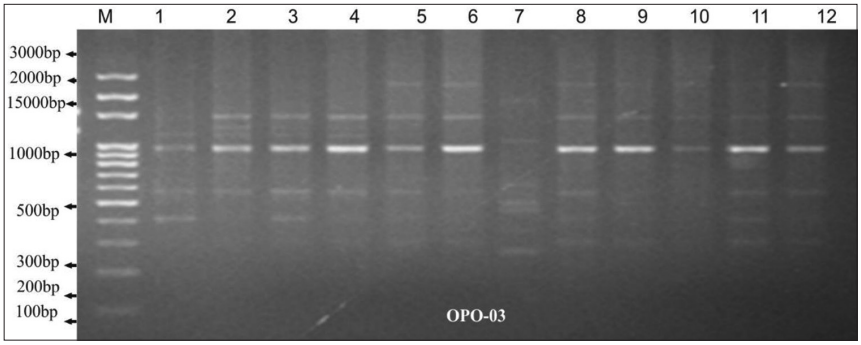

Fig 8. RAPD-PCR analysis of 12 linseed accessions with OPO-03Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

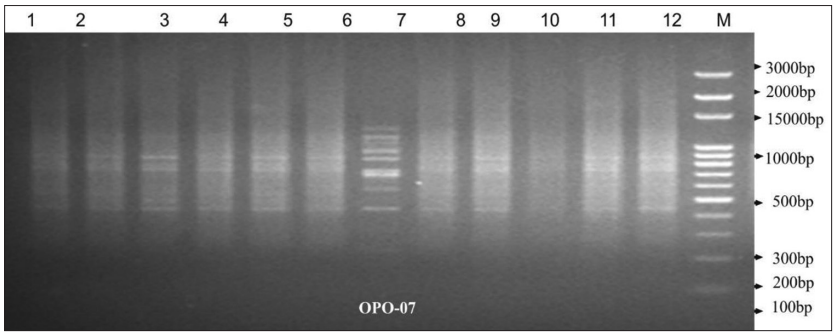

Fig 9. RAPD-PCR analysis of 12 linseed accessions with OPO-07 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

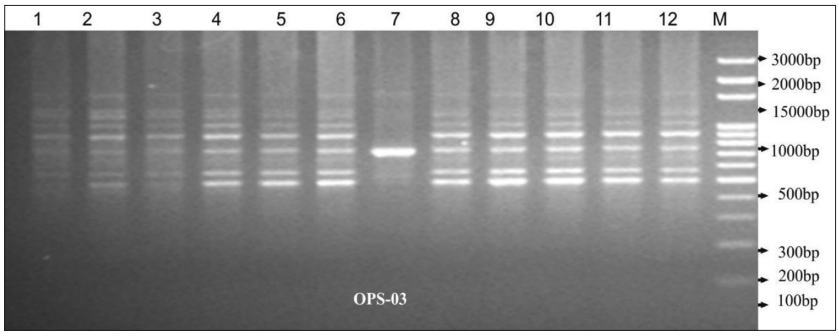

Fig 10. RAPD-PCR analysis of 12 linseed accessions with OPS-03 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

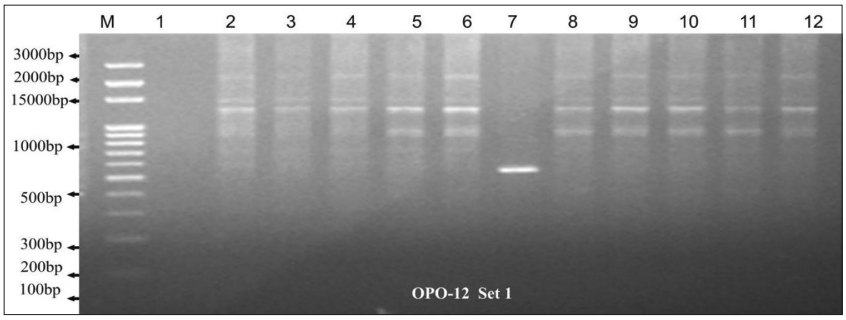

Fig 11. RAPD-PCR analysis of 12 linseed accessions with OPO-12 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

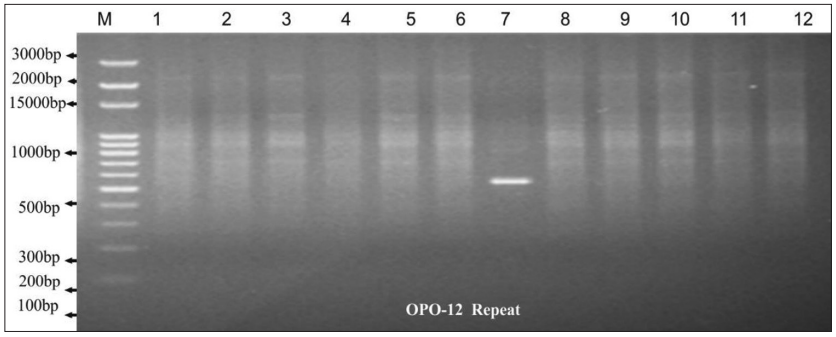

Fig 12. RAPD-PCR analysis of 12 linseed accessions with OPO-12 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

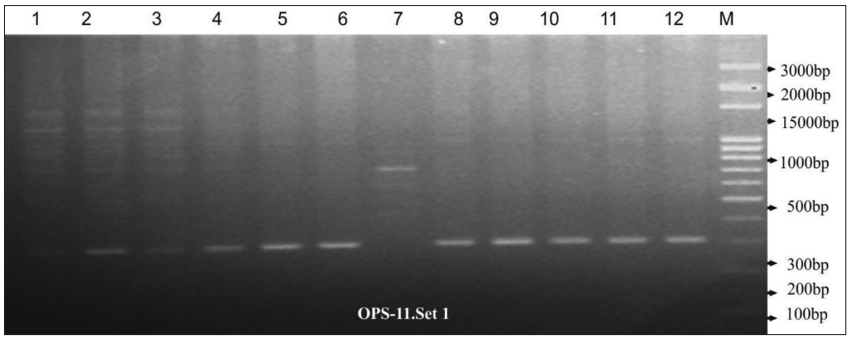

Fig 13. RAPD-PCR analysis of 12 linseed accessions with OPS-11 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

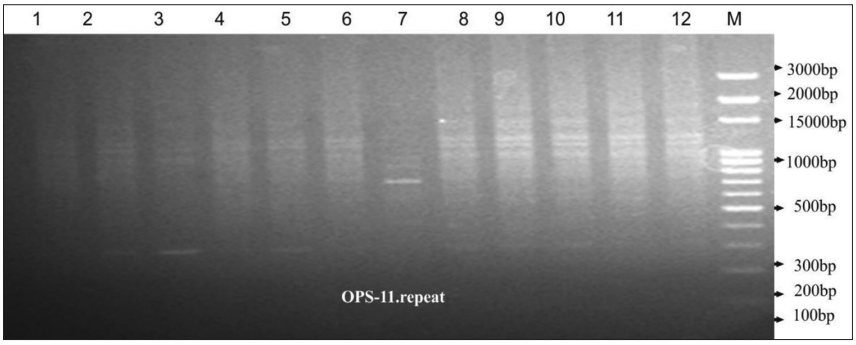

Fig 14. RAPD-PCR analysis of 12 linseed accessions with OPS-11 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

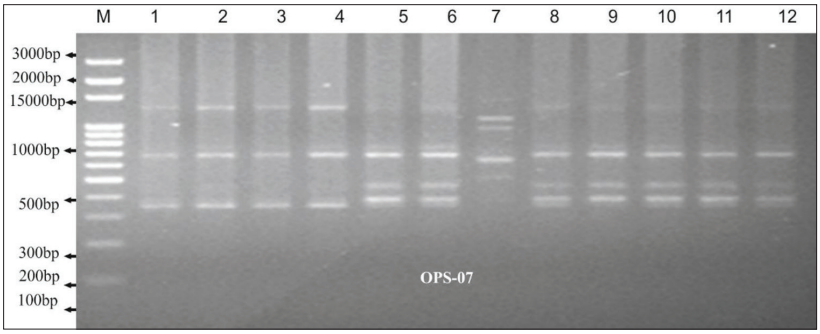

Fig 15. RAPD-PCR analysis of 12 linseed accessions with OPS-07 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

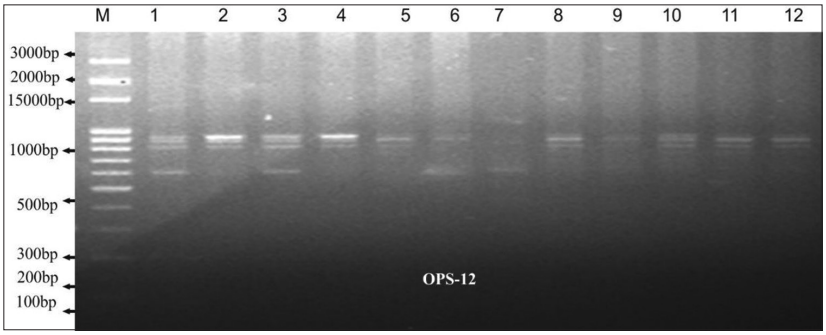

Fig 16. RAPD-PCR analysis of 12 linseed accessions with OPS-12 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

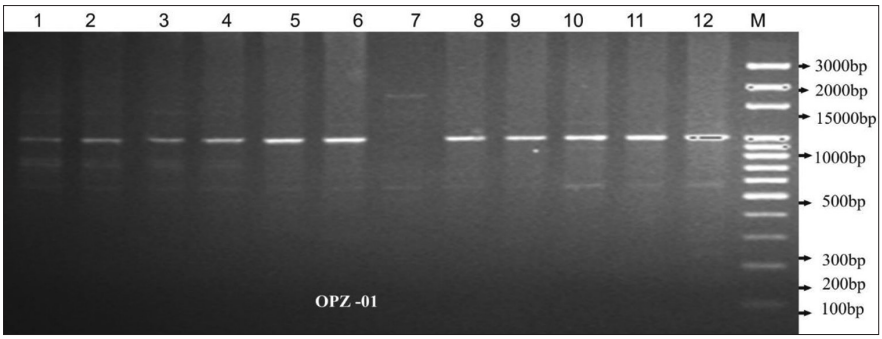

Fig 17. RAPD-PCR analysis of 12 linseed accessions with OPZ-01 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

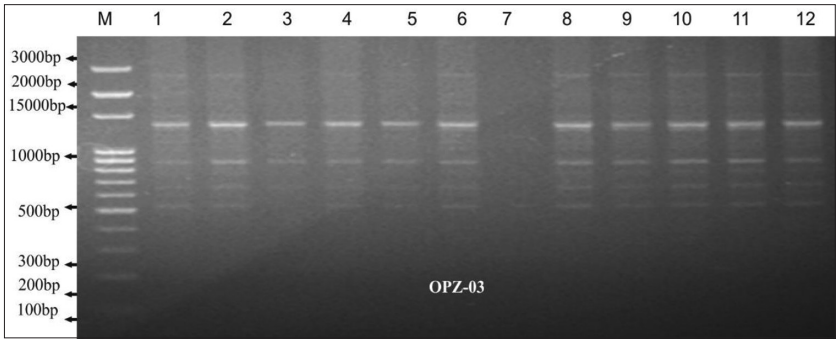

Fig 18. RAPD-PCR analysis of 12 linseed accessions with OPZ-03 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

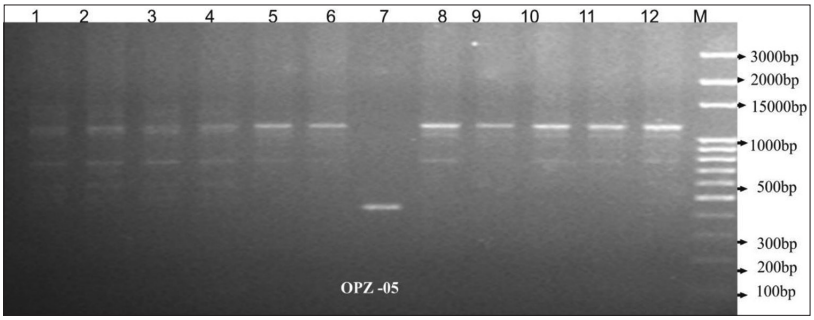

Fig 19. RAPD-PCR analysis of 12 linseed accessions with OPZ-05 Random primer. Lane M =DNA lader.Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

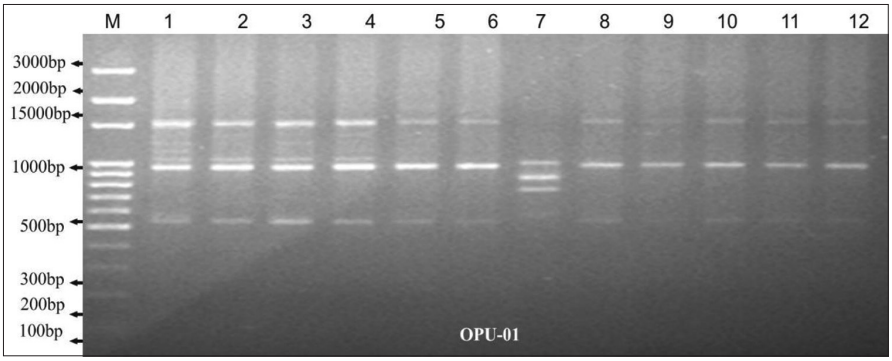

Fig 20. RAPD-PCR analysis of 12 linseed accessions with OPU-01 Random primer. Lane M =DNA lader. Lane:1= IC 564616, Lane2= 564622, Lane:3= IC 564631, Lane:4= IC 564592, Lane:5= IC 564630, Lane:6= IC 564660, Lane:7= IC 564585, Lane:8= IC 564676, Lane:9= IC 564624, Lane:10= IC 564605, Lane:11= BHU-A, Lane:12=BHU-B.

Table 3: Genetic similarity derived from Jaccard's coefficient among 12 linseed accessions

	IC 564616	IC-564622	IC-564631	IC-564592	IC-564630	IC-564660	IC-564585	IC-564676	IC-564624	IC-564605	BHU-A	BHU-B
IC-564616	1.0000000											
IC-564622	0.8947368	1.0000000										
IC-564631	0.9122807	0.9137931	1.0000000									
IC-564592	0.8793103	0.9473684	0.8983051	1.0000000								
IC-564630	0.7049180	0.7377049	0.7258065	0.7833333	1.0000000							
IC-564660	0.7096774	0.7419355	0.7301587	0.7868852	0.9259259	1.0000000						
IC-564585	0.1267606	0.1081081	0.1216216	0.1066667	0.0985915	0.0958904	1.0000000					
IC-564676	0.7213115	0.7833333	0.7419355	0.8000000	0.9074074	0.9444444	0.0821918	1.0000000				
IC-564624	0.7213115	0.7833333	0.7419355	0.8305085	0.9433962	0.9090909	0.0972222	0.9259259	1.0000000			
IC-564605	0.7258065	0.7868852	0.7460317	0.8333333	0.9444444	0.9454545	0.0945946	0.9619630	0.9619630	1.0000000		
BHU-A	0.7833333	0.7868852	0.8032787	0.8032787	0.9090909	0.8771930	0.0945946	0.9272727	0.8928571	0.9285714	1.0000000	
BHU-B	0.7540984	0.8166667	0.7741935	0.8333333	0.9090909	0.9107143	0.0945946	0.9619630	0.9272727	0.9626364	0.9636364	1.0000000

DISCUSSION

Molecular markers are widely used to assess genetic variations and utilized to evaluate genetic differences among accessions as well as to investigate seed clarification, assessment of genotypes and marker aided breeding (Baack et al., 2005; Paniego et al., 1999; Barbara et al., 2007). Germplasm variability is important to accomplish various objectives of crop including enhanced yield, desirable quality, broader adaptability, diseases and pests resistance (Begum et al., 2007). The scope and type of the correlation between characters allow for developing the multiple trait selection methods efficiently. Furthermore, understanding the naturally happening variations in a population aids in the identification of various genotype groups (Tadesse et al., 2009).

As compared to previous reports, the percentages of polymorphisms in this analysis were higher (75% to 100 %) with an average of 93.15. Abou El-Nasar et al., (2014) examined at five different linseed accessions and found that the polymorphic DNA bands formed by six RAPD primers were 27 out of 56, with a percentage of (48.2). One other RAPD analysis against linseed accessions reported (63.06%) polymorphism (Prabhakar Kumar singh et al., 2009). According to Fu et al., (2003) RAPD differences in linseed accessions were poor since there were just one or two polymorphic bands per primer. In contrast, in our study RAPD displayed a higher level of polymorphism and a significant number of distinctly amplified bands between accessions.

Our findings indicated that the markers utilized in current investigation were very informative and 16 RAPD primers produced an average of 5.7 bands which were consistent to that identified in a previous study by (Prabhakar Kumar singh et al., 2009; Kumari, et al., 2017) who obtained mean RAPD bands of 5.5 and 4.5 respectively in various linseed accessions. The mean PIC value (0.45) obtained in this study was higher as compared to (0.387) in linseed genetic diversity analysis using RAPD and ISSR markers (Kumari, A. et al., 2017) and (0.30) in flax germplasm using SSR markers (Soto-Cerda et al., 2011).

The value of genetic similarity generated in this study was ranging from 8.2 to 96.3%. Whereas in others studies varied from 60% to 70% (Abou El-Nasar and Mahfouze, 2013) and 18 to 90% (Bhavita et al., 20121). Chandrawati et al., (2014), reported genetic similarity values in linseed accession through AFLP between 16% to 57%. Our results were supported by Ziarovska et al., (2012) and Fu et al., (2005) who reported that the world collection of cultivated linseed contains a high level of genetic diversity.

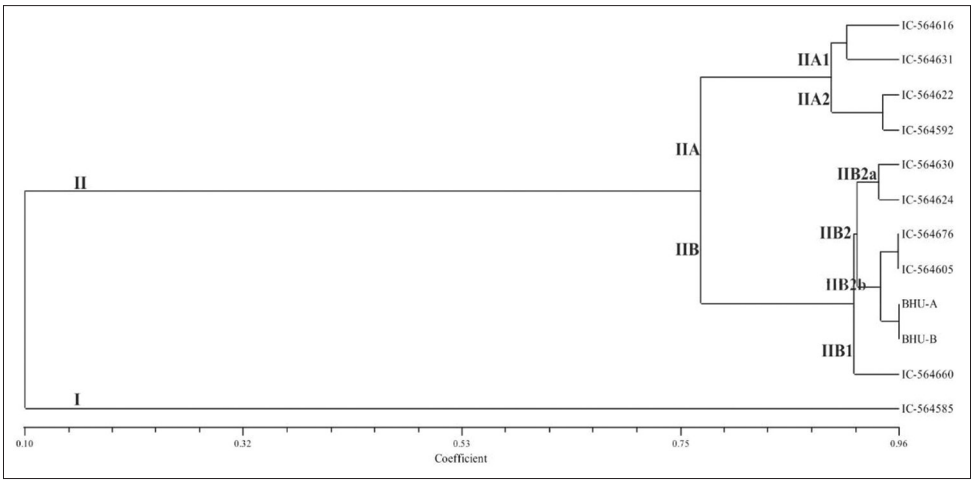

Fig 20. Dendrogram pattern of RAPD analysis of 12 linseed accessions with UPGMA (Unweighted Pair Group Method with Arithmetic average). IC 564616, 564622, IC 564631, IC 564592, IC 564630, IC 564660, IC 564585, IC 564676, IC 564624, IC 564605, = BHU-A, BHU-B.

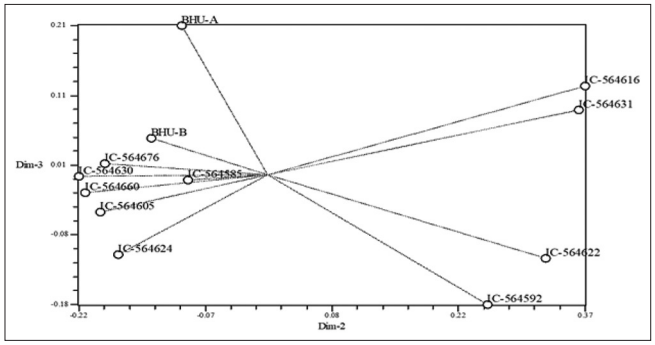

Fig 21. 2D Dendrogram of 12 linseed accessions.

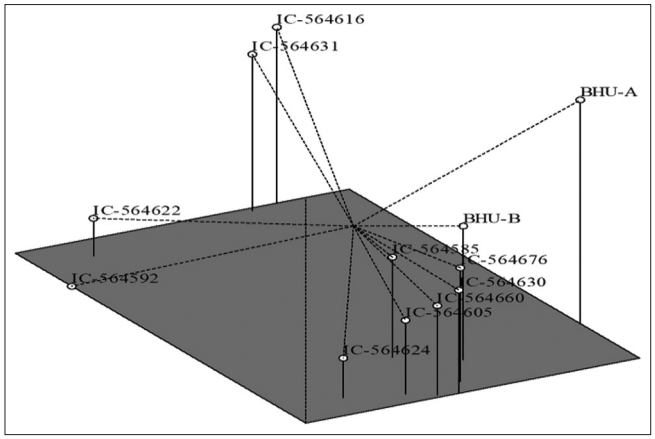

Fig 22. 3D dendrogram of 12 linseed accessions.

CONCLUSION

In the current analysis, the genetic variation of 12 linseed accessions is estimated using 16 RAPD markers. RAPD data indicated that polymorphism ranged from 75% to100%, with an average of 93.15% across all accessions. The genetic similarity was between 8.2% and 96.3%. Based on similarity and cluster analysis IC 564585 accession was found to be more divergent followed by IC 564616, IC 564631, IC 56422, IC 564630 and these can be used as linseed crop improvement. The genetic similarity obtained from the analysis can also be used for the selection of parents to generate mapping population and for selecting parents for breeding purposes. Result of this study suggests that RAPD primers are capable of measuring polymorphism, similarities and accessions differentiation using unique RAPD markers for linseed accessions. Finally, the present research provides valuable information for breeders to establish linseed accessions resources and their subsequent usage in breeding programs.

ACKNOWLEDGMENTS

Authors would like to thank UGC-BSR Start-up Grant for supporting the project financially and one of us (Beema Nagabhushanam) expresses gratitude to the Council of Scientific & Industrial Research (CSIR), New Delhi, for awarding a Junior Research Fellowship to him. Grateful to the National Bureau of Plant Genetic Resources (NBPGR) and Banaras Hindu University (BHU) in Varanasi, Uttar Pradesh, India, for providing linseed germplasm.

Author's contribution

BN: Conducted the experiment, assisted in data analysis; MIM, MN: Conducted the experiment; ES, DR: Data analysis; KK: Collected samples, Designed the experiment and draft manuscript preparation. The final manuscript was read and accepted by all authors.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

Abou El-Nasar, T. H. S., M. S. Hassanein, M. E. S. Ottai and M. A. Al-Kordy. 2014. Genetic diversity among five Romanian linseed varieties under Egyptian conditions. Middle East J. Appl. Sci. 4: 114-121.

Abou El-Nasar, T. H. S. and H. A. Mahfouze. 2013. Genetic variability of golden flax (*Linum usitatissimum* L.) using RAPD markers. World Appl. Sci. J. 23: 851-856.

Baack, E. J., K. D. Whitney and L. H. Rieseberg. 2005. Hybridization and genome size evolution: Timing and magnitude of nuclear DNA content increases in *Helianthus* homoploid hybrid species. New Phytol. 167: 623-623.

Barbara, T., C. Palma-Silva, G. M. Paggi, F. Bered, M. F. Fay and C. Lexer. 2007. Cross-species transfer of nuclear microsatellite markers: Potential and limitations. Mol. Ecol. 16: 3759-3767.

Begum, H., A. K. M. Alam, M. J. A. Chowdhary and M. I. Hossain. 2007. Genetic divergence in linseed (*Linum usitatissimum* L.). Int. J. Sustain. Crop. Prod. 2: 4-6.

Bhavita, G., J. Vaishanavi and S. D. Mahendra. 2021. Assesment of genetic diversitu and physic-chemical analysis of linseed (*Linum usitatissimum* L.) genotypes. Pharm. Innov. 10: 328-331.

Bjelkova, M., J. Nozova, K. Fatrcova-Sramkova and E. Tejklova. 2012. Comparision of linseed (*Linum usitatissimum* L) genotype with respect to the content of polyunsaturated fatty acids. Chem. Pap. 66: 972-976.

Chandrawati, R. Maurya, P. K. Singh, S. A. Ranade and H. K. Yadav. 2014. Diversity analysis in Indian genotypes of linseed (*Linum usitatissium* L.) using AFLP markers. Gene. 549: 171-178.

Diederichsen, A. and J. P. Raney. 2006. Seed colour, seed weight and seed oil content in *Linum usitaitissimum* accessions held by plant gene resources of Canada. Plant Breed. 125: 372-373.

Doyle, J. J. and J. E. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 9: 11-15.

El-Beltagi, H. Z. A., D. M. Salama and El-Hariri. 2007. Evaluation of fatty acids profile and the content of some secondary metabolites in seeds of different flax cultivars (Linum usitatissimum L). Gen. Appl. Plant Physiol. 33: 187-202.

Fu, Y. B. 2005. Geographic patterns of RAPD variation in cultivated flax. Crop Sci. 45: 1084-1091.

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: 1084-1091.

Hosseinian, F. S., G. G. Rowland, P. R. Bhirud, J. H. Dyck and R. T. Tyler. 2004. Chemical composition and physicochemical and hydrogenation characteristics of high-palmitic acid solin (low-linolenic acid flaxseed) oil. J. Am. Oil Chem. Soc. 81: 185-188.

Jaccard, P. 1908. Nouvelles researches la distribution florae. Bull. Soc. Vaud. Sci. Nat. 44: 223-270.

Kumar, A., S. Paul and V. Sharma. 2018. Genetic diversity analysis using RAPD and ISSR Markers revealed discrete genetic makeup in relatonto fibre and oil content in (*Linum usitatissimum* L.) genotypes. Nucleus. 61: 45-53.

Mansby, E., O. Diaz and R. V. Bothmer. 2000. Preliminary study of genetic diversity in Swedish flax (*Linum usitatissimum* L). Resources Crop Evol. 47: 417-424.

Paniego, N., M. Echaide, M. Munoz, L. Fernandez, S. Torales, P. Faccio, I. Fuxan, M. Carrera, R. Zandomeni, E. Y. Suarez and H. E. Hopp. 2002. Microsatellite isolation and characterization in sunflower (*Helianthus annuus* L.) Genome. 45: 34-43.

Patzk, J. 2001. Comparison of RAPD, STS, ISSR and AFLP molecular methods used for assessment of genetic diversity in hop (*Humulus lupulus* L.). Euphytica. 121: 9-18.

Prabhakar, K. S., M. Akram and R. L. Srinivas. 2009. Genetic Diversity in linseed (*Linum usitatissimum* L) cultivars based on RAPD analysis. Indian J. Agric. Sci. 79: 1046-1049.

Ragupathy, R., R. Rathinavelu and S. Cloutier. 2011. Physical mapping and BAC-end sequence analysis provide initial insights into the flax (*Linum usitatissimum* L.) genome. BMC Genomics. 12: 217.

Rohlf, F. J. 2000. NTSY-pc Numarical Taxonomy and Multivariate Analysis System. Version.2.02. Exeter Software, Setauket, New York.

Roldan-Ruiz, I., J. J. Dendauw, V. Bockstaele, A. Depicker. M. Loose. 2010. AFLP Markers reval high poly morphic rates in ryegrasses (*Lolium* spp.). Mol. Breed. 6: 125-134.

Saeidi, G. 2012. Genetic variation and heritability for germination, seed vigour and field emergence in brown and yellow- seeded genotypes of flax. Int. J. Plant Prod. 2: 15-22.

Schmidt, T. J., M. Klaes and J. Sendker. 2012. Lignans in seeds of *Linum* species. Phytochemistry. 82: 89-99.

Soto-Cerda, B. J., H. U. Savedra, C. N. Navarro and P. M. Ortega. 2011 Characterization of novel genic SSR markers in *Linum usitatissimum* (L.). and their transferability across eleven *Linum* species. Electron. J. Biotechnol. 14: 6.

Srivastav, V. S., C. V. Kapdia, M. K. Mahatma, S. K. Jha and T. Ahmed. 2013. Genetic diversity analysis of date palm (*Phoenix dactylifera* L.) in the Kutch Region of India using RAPD and ISSR markers. Emir. J. Food Agric. 25: 907-915.

Tadesse, T., H. Singh and B. Weyessg. 2009. Genetic divergence in linseed Germplasm. J. Innov. Dev. Strategy. 3: 13-20.

Ulloa, O., F. Ortega and H. Campos. 2003. Analysis of genetic diversity in red clover (*Trifolium pararnse* L.) breeding populations as revealed by RAPD genetic markers. Genome. 46: 529-535.

Umesh, R. P., J. Baskaran, I. P. Ajithkumar, and R. Pannerselvam. 2013. Genetic variation between *Xylocarpus* spp. (Meliaceae) as revealed by random amplified polymorphic DNA (RAPD) markers. Emir. J. Food Agric. 25: 597-604.

Westcott, N. A. and A. D. Muir. 2003. Flax seed lignin in disease prevention and health promotion. Phytochem. Rev. 2: 401-417.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey. 1990. Amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531-6535.

Yadava, D. K., S. Vasudev, N. Singh, T. Mohapatra and K.V. Prabhu. 2012. Breeding major oil crop: Present status and future research needs. In: Technological Innovations Major World Oil Crops Breed. Vol. 1. pp. 17-51.

Ziarovska, J., K. Razna, S. Senkova, V. Stefunova and M. Bezo. 2012. Variability of *Linum usitatissimum* L. based on molecular markers. ARPN J. Agric. Biol. Sci. 7: 50-58.

Zohary, D. 1999. Monophyletic and polyphyletic orgin of the crops on which agriculture was formed in the near East. Genet. Res. Crop Evol. 46: 133-142.