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ESTIMATION OF GENETIC DIVERSITY AMONG *LINUM USITATISSIMUM* L. GERMPLASM THROUGH MOLECULAR MARKERS

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Abstract

The present study was undertaken to evaluate heritable diversity of 42 linseed (*Linum usitatissimum* L.,) germplasm, 30 from the Pakistan and 12 from the USA through molecular techniques. The research was performed at the National Institute of Genomics and Biotechnology (NIGAB), NARC Islamabad Pakistan. For this purpose, twenty molecular markers (13 ISSR and 7, SSR) has been chosen for the assessment of diversity amongst forty two linseed genotypes through DNA-PCR based technique. DNA extraction was carried out by using the CTAB method. Out of twenty molecular markers (13 ISSR and 7 SSR), sixteen markers were found to be reproducible while four markers (2 of ISSR and SSR each) were not reproducible. Molecular data was demonstrated by dendrograms generated through UPGMA method based on bivariate data matrix. Highly branched clusters based on genetic associations among genotypes were presented by ISSR markers as compared to SSR markers. The 100 to 400 bp fragments were amplified by the SSR markers profiling while 100-1100bp in ISSR profiling. The most significant and diverse genotypes found through SSR markers were 021750, 021570, 021245, 021425 and 021310 while only 1 most diverse genotype 021570 was found through ISSR markers. Hence the genotypes 020885, 020890 and 020870 are recommended for their further used in breeding programs while mapped markers should be used for gene sequencing of genotype 021570.

Key words: *Linum usitatissimum,* Genetic diversity, ISSR, SSR, Markers.

Introduction

Flax or Lineseed belonging to genus Linum, also known as "Alsi" and having chromosome numbers 2n=30. Flax has been cultivated for thousands of years because of its oil and good quality of its stem fiber (Pali and Mehta, 2016). The highest rate of linseed production was recorded in three countries of the world; Canada (23%), China (21%) and 14% by Russia (Anon., 2013). The growth of linseed in Pakistan is carried out in Sindh and Punjab (PARC, 2009). *Linum usitatissimum* L. is of countless importance in human nutrition because of its active and beneficial components. Its seeds can be consumed by human being in different ways; either crushed or powdered to add in bakery products or in oil form (Bernacchia, Preti & Vinci, 2014). The nutritional profile of flaxseed shows its significant health supporting properties. Its seeds are considered to be the healthy source of oil which contains digestible proteins, poly-unsaturated fatty acids and lignin (Akhter *et al.,* 2013). In Pakistan, linseed is cultivated for oil purposes which is extracted from its seeds and after `extracting fiber from its stem, remaining material can be crushed and used for the manufacturing of straw board and paper (Gul *et al.,* 2016). ISSR based genetic diversity evaluation is a prevalent technique to sequence the un-sequenced genes and is mostly in use for evolution studies and crop genetics evaluation (Zietkiewicz *et al*., 1994). Different types of techniques have been developed to study the crop improvement, which includes genetic diversity assessment and germplasm characterization. SSRs have been largely used for the genetic studies due to their remarkable characteristics like co-dominance, polymorphic ability, reproducibility and abundance (Kulueng *et al*., 2004 and Xie *et al*., 2011). Wiesner & Iesnerova (2003) optimized the ISSR technique for flax fingerprinting. 70 Indian flax genotypes were analyzed by Rajwade *et al*., (2010) for genetic association based on PCR based ISSR primers. Evaluation of crops with molecular markers is rapid and accurate cultivar documentation, which has shown a robust method for genome classification of crops, collection and organization. SSR markers are being broadly used crops genetics analysis and identification because of their codominnce and polymorphisim (Kulueng *et al*., 2004 and Xie *et al*., 2011). The aim of the study was to evaluate the genetic diversity of *Linum usitatissimum* L gemplasm belonging to both the Pakistan and the America by using SSR ISSR markers.

Materials and Method

The study was carried out for the estimation of genetic diversity among linseed (*Linum usitatissimum* L.) germplasm at National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agriculture Research Center (NARC), Islamabad Pakistan.

Plant Material: The seeds of linseed (*Linum usitatissimum* L.) genotypes were collected from Plant Genetic Research Institute (PGRI), department of National Agricultural Research Centre (NARC), Islamabad Pakistan. Total 42 genotypes of *Linum usitatissimum* L., were used, of which 32 genotypes were from Pakistan and 10 from America. These were sown and harvested in the field of National Institute for Genomics and Advanced Biotechnology at NARC, Islamabad. List of linseed germplasm used for present research work was shown in Table 1.

Table 1. List of Linseed genotypes used for the assessment of genetic diversity.

Sr. No.	Genotypes	Origin	Location
1.	020870	Pakistan	Lahore
2.	020885	Pakistan	Islamabad
3.	020886	Pakistan	Islamabad
4.	020887	Pakistan	Islamabad
5.	035151	Exotic	USDA
6.	020889	Pakistan	Islamabad
7.	020890	Pakistan	Islamabad
8.	020891	Pakistan	Islamabad
9.	020893	Pakistan	Islamabad
10.	035126	Exotic	USDA
11.	021160	Pakistan	Faisalabad
12.	021195	Pakistan	Faisalabad
13.	021200	Pakistan	Faisalabad
14.	021245	Pakistan	Jhang
15.	021310	Pakistan	Jhang
16.	021400	Pakistan	Muzaffargarh
17.	021425	Pakistan	Attock
18.	021570	Pakistan	T.T. Singh
19.	021750	Pakistan	Karachi
20.	021890	Pakistan	Sargodha
21.	021920	Pakistan	Lahore
22.	022305	Pakistan	Rawalpindi
23.	035011	Exotic	USDA
24.	035147	Exotic	USDA
25.	035031	Exotic	USDA
26.	035032	Exotic	USDA
27.	035037	Exotic	USDA
28.	035146	Exotic	USDA
29.	035016	Exotic	USDA
30.	035127	Exotic	USDA
31.	020888	Pakistan	Islamabad
32.	031951	Pakistan	Hasilpur 5
33.	035880	Pakistan	Hunza
34.	031542	Pakistan	Sheikhupura
35.	020900	Pakistan	Lahore
36.	031538	Pakistan	Hasilpur 1
37.	036756	Pakistan	Ghizer
38.	031540	Pakistan	Hasilpur 3
39.	028359	Pakistan	Mianwali
40.	031539	Pakistan	Hasilpur 2
41.	031541	Pakistan	Hasilpur 4
42.	035881	Pakistan	Hunza

Molecular characterization of linseed (*Linum usitatissimum* L.): At molecular level 20 molecular markers (13 ISSR and 7 SSR markers) were designed for evaluating the genetic diversity of these selected genotypes by recording the presence and absence of bands (Tables 2,3).

DNA extraction and PCR amplification: DNA extraction was carried out from fresh and healthy leaves of all selected samples through CTAB method as described by Doyle & Doyle (1990). Firstly, CTAB heated in water bath for 30 minutes. Then leaf samples were grinded in mortar and pestle by using 600µl of heated CTAB. Then fine extract was poured in eppendorf tubes and were put in water bath at 65°C for 45 minutes. After heating 750µl of Chloroform isomylalcohol was added in the tubes and then centrifuged at 12,000 rpm for 10 minutes. Afterwards supernatant was taken and added in new labeled eppendorf tubes. About 480µl of chilled Isopropanol was added in them and for the precipitation of DNA these tubes were incubated at -4°C for 10 minutes. After this, tubes were centrifuged at 12,000 rpm for 10 minutes. The liquid from these tubes was discarded and 100µl of 75% Ethanol was added in them and centrifuged for ten minutes at 12 thousands rpm to wash the pellet from debris. Subsequently, liquid was discarded and along with pellet these tubes were left for overnight to dry. Next day 100µl of double distilled water was added in tubes so as to make DNA sample. Then DNA samples were detected by 1% agarose gel suspended in gel tank 1× TBE buffer and gel electrophoresis was set at 100 voltages for 30-40 minutes for running of this gel. Then Gel Documentation System was used for the visualization of DNA present in these samples. 20 molecular markers (13 ISSR and 7 SSR markers) were screened and amplified for this study. For the preparation of Master Mix 20ul volume of Polymerase chain reaction (PCR) was prepared. Reaction mixture consist of 1× Taq buffer having $(NH_4)_2SO_4$, 3mM of $MgCl_2$, 0.2mM of dNTPs mix, 1.8 µl of Taq polymerase, 10pmol of forward and reverse primers, 1 µl of DNA concentration. Applied Biosystems Thermal Cycler was used for PCR amplification at 94ºC for three (3) minutes. It consists of 3 steps; first step is of denaturation which was at 94ºC for three (3) minutes followed by 40 cycles for 40 seconds and second step primer annealing was for 1 minute at 72ºC. Extension period is at 72ºC for 10 minutes. The PCR profile described denaturation, annealing and extension temperature of each ISSR and SSR marker at which they amplified whole population. Resolution of PCR amplified product was carried out at 1.5 to 2% of agarose gel having 1 Kb DNA ladder through the use of UV Trans-illuminator to illuminate the bands found in it so to identify and record these bands.

Data analysis

The recorded molecular data was evaluated through bivariate data matrix in which all bands were counted by zero and one. By using UPGMA method, dendrograms were generated to assess genetic associations among linseed genotypes. The PIC content of both ISSR and SSR primers was calculated and similarity matrix of genotypes in cluster analysis was constructed through computer software NTSyS.PC2.1.

Table 2. List of SSR Primers with their PCR profile used for the estimation of genetic diversity in linseed genotypes.

Sr. No.	Primers	Sequence (5`- 3`)	PCR Profile
1.	LU 8	F:TCCCGTAATATTCTATGTTCTTCC R:TGAGTTGGACCTTACAAGACTCA	94°C: 3 min, 1 cycle 94°C: 40s, 46°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
2.	LU 10	F:GCCTAAAGCTGATGCGTTTC R:TGTCAGGCTCCTTCTTTTGC	94°C: 3 min, 1 cycle 94°C: 40s, 49°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
3.	LU 11	F:ATGGCAGGTTCTGCTGTTTC R:TTGCGTGATTATCTGCTTCG	94°C: 3 min, 1 cycle 94°C: 40s, 48°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
4.	LU 24	F:ATGGCAGGTTCTGCTGTTTC R:TTGCGTGATTATCTGCTTCG	94°C: 3 min, 1 cycle 94°C: 40s, 49°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
5.	LU 25	F:TCTACAGAGTTCAATTCCCGTAA R:GTTGGACCTTACAAGACTCACTG	94°C: 3 min, 1 cycle 94°C: 40s, 49°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
6.	gwm 637	F: AAAGAGGTCTGCCGCTAACA R: TATACGGTTTTGTGAGGGGG	94°C: 3 min, 1 cycle 94°C: 40s, 49°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
7.	gwm 149	F: CTAGCATCGAACCTGAACAAG R: CATTGTTTTCTGCCTCTAGCC	94°C: 3 min, 1 cycle 94°C: 40s, 49°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle

Table 3. List of ISSR Primers with their PCR profile used for the estimation of genetic diversity in linseed genotypes.

Sr. No.	Primers	Sequence (5`- 3`)	PCR Profile
1.	UBC-810	GAGAGAGAGAGAGAGAT	94°C: 3 min, 1 cycle 94°C: 40s, 43°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
2.	UBC-818	CACACACACACACACAG	94°C: 3 min, 1 cycle 94°C: 40s, 52°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
3.	UBC-825	ACACACACACACACACT	94°C: 3 min, 1 cycle 94°C: 40s, 36°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
4.	UBC-840	GAGAGAGAGAGAGAGAYT	94°C: 3 min, 1 cycle 94°C: 40s, 43°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
5.	UBC-855	ACACACACACACACACYT	94°C: 3 min, 1 cycle 94°C: 40s, 49°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
6.	ISSR 1	AGAGAGAGAGAGAGAGT	94°C: 3 min, 1 cycle 94°C: 40s, 53°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
7.	ISSR 2	AGAGAGAGAGAGAGAGC	94°C: 3 min, 1 cycle 94°C: 40s, 51°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
8.	ISSR 3	AGAGAGAGAGAGAGAGG	94°C: 3 min, 1 cycle 94°C: 40s, 52°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
9.	ISSR 4	AGAGAGAGAGAGAGAGT	94°C: 3 min, 1 cycle 94°C: 40s, 51°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
10.	ISSR 5	GAGAGAGAGAGAGAGAC	94°C: 3 min, 1 cycle 94°C: 40s, 51°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
11.	ISSR 6	GAGAGAGAGAGAGAGAA	94°C: 3 min, 1 cycle 94°C: 40s, 51°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
12.	ISSR 815	CTCTCTCTCTCTCTCTGT	94°C: 3 min, 1 cycle 94°C: 40s, 48°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle
13.	ISSR 819	GTGTGTGTGTGTGTGTA	94°C: 3 min, 1 cycle 94°C: 40s, 48°C: 40s, 72°C: 1min 40 cycles 72°C 10min 1 cycle

Results

Seven SSR primer pairs applied in this study were LU 8, LU 10, LU 11, LU 24, LU 25, gwm 637 and gwm 149 among 42 genotypes of *Linum usitatissimum* L. Out of 7 primers, 5 were amplified PCR product and 2 markers gwm 637 and gwm 149 did not amplify a single fragment in any accession hence these 2 were excluded. The annealing temperature for all primers was optimized by providing different temperature ranges. The amplified fragments were matched with 100 –bp DNA ladder and data was recorded both on bivariate and multivariate data matrix. In bivariate 0 and 1 was used for the presence and absence bands and for multivariate data complex. SSR primers produced 235 total bands of which LU 8, LU 10, LU 11, LU 24 and LU 25 generated 27, 39, 36, 96 and 37 amplified fragments. Out of 42 genotypes, 38 genotypes gave results while 4 genotypes gave no bands in this amplified PCR product. 24 genotypes amplified a fragment of 190 base pair while 9 genotypes amplified fragments of 180bp, 3 genotypes amplified fragment of 170 bp and 1 genotype produced the fragment of 200 bp and 100 bp each. For LU 10 primer 35 genotypes showed their reproducible bands at 190 bp while 7 genotypes amplified the fragment of 200 bp. LU 24 was recorded for polymorphic bands in linseed accessions. PCR product amplified fragment were ranged between 100bp-400bp (Figs. 1 and 2).

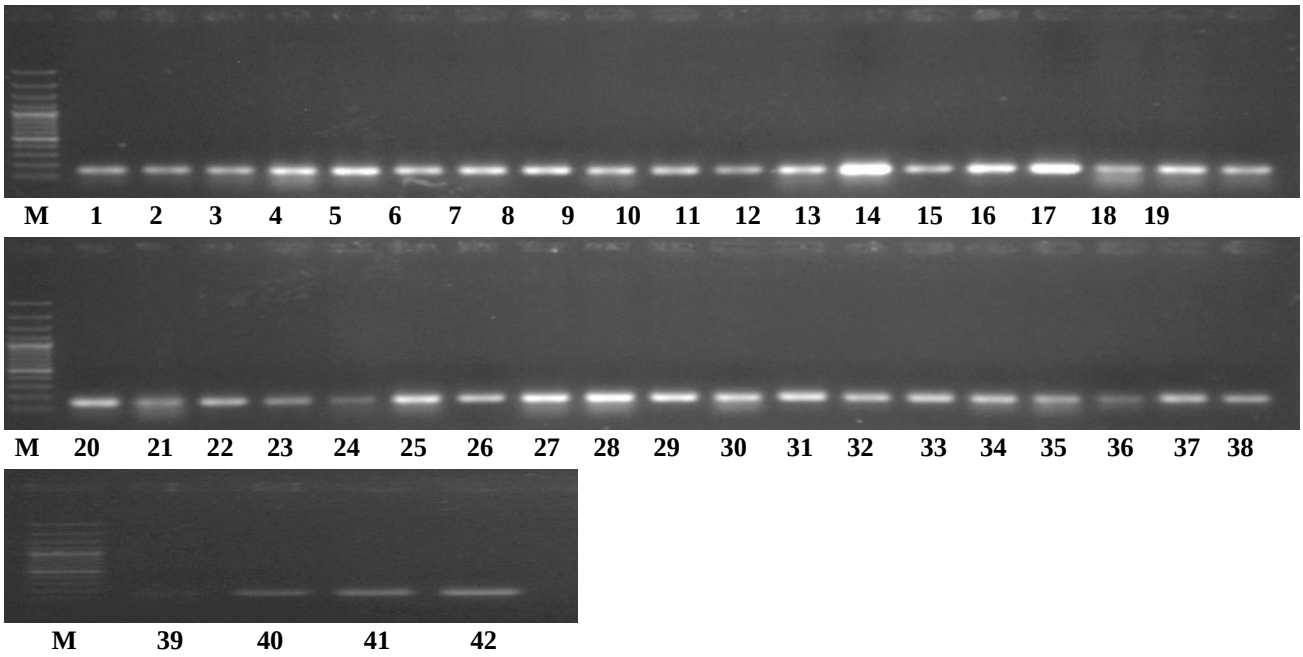

Fig. 1. Gel picture for LU 10 SSR marker, DNA ladder 100 bp.

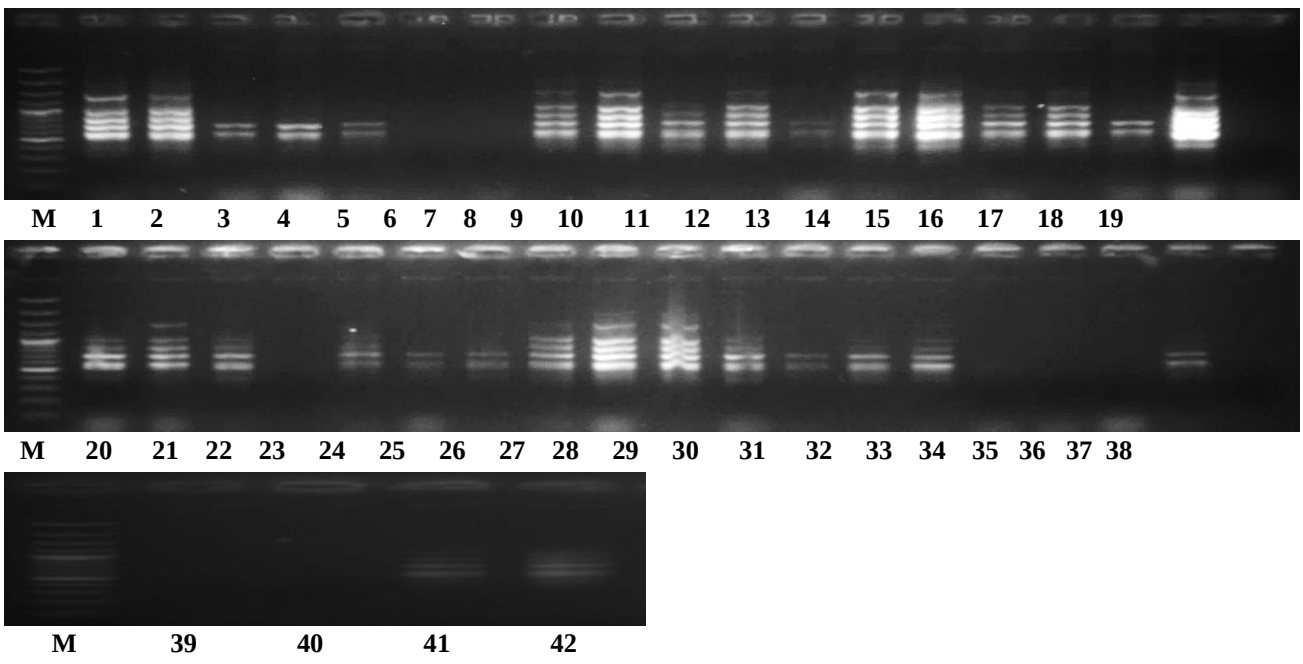

Fig. 2. Gel picture for UBC 810 ISSR marker, DNA ladder 100 bp.

The multivariate analysis was conducted to form a dendrogram of similarity index based on UPGMA (Using Pair group method of arithmetic means) to evaluate the genetic similarity and difference among linseed genotypes (Fig. 3). The dendrogram is divided into two main groups A and B. Genotypes present in these two groups showed maximum genetic diversity from each other. Out of 42 genotypes, 19 genotypes (45.2%) were present in cluster A-1-1 and cluster A-1-2 comprised of 3 genotypes (7.1%) whereas cluster A-2-1 and A-2-2 contained only 2 (4.7%) and 1 (2.3) genotype, respectively. The sub-group B-1 further categorized into 2 clusters, showing 64% genetic similarity with each other (Fig. 3). The sub-group B-2 categorized into 3 clusters which consisted of maximum number of genetically similar genotypes (45.2% and 21.4%) while 2 genotypes viz., 315380 and 21920 observed to be more diverse genotypes (Table 4). The cluster B-1-1 contain 19 genotypes i.e. 20885 (Pak), 21310 (Pak),20891 (Pak), 21160 (Pak), 35126 (USDA), 21200 (USDA), 21245 (Pak), 21890 (Pak), 20886 (Pak) ,31951 (Pak), 35880 (Pak), 21425 (Pak),35031 (USDA), 35147 (USDA), 35032 (USDA) ,35037 (USDA), 35146 (USDA), 35016 (USDA) and 35127 (USDA). Genotypes 20893 (Pak), 21920 (Pak), 20870 (Pak), 21195 (Pak) and 35151 (USDA) were present in cluster B-1-2.

Evaluation of genetic diversity among genotypes of linseed through ISSR markers: Out of 13 primers, only 11 produced score able bands therefore these were selected for molecular analysis and the remaining 2 markers (ISSR 6 and ISSR 815) failed to produce any band. The ISSR primers produced 1296 amplified fragments for the studied genotypes. The number of bands produced by these markers were 139, 279, 103, 155, 142, 138, 178, 7, 10, 04.

The PIC value (Polymorphic information content) of molecular marker showed polymorphism of marker that ranged from 0-1 and greater value depict more variance in alleles of studied population. In present study, the polymorphic information content value of each ISSR marker was calculated through following formula:

$$PIC_j = 1 - \sum_{i=1}^{n} Pi^2$$

The multivariate analysis was conducted for the formation of dendrogram (Fig. 4) through UPGMA method and formed two main groups A and B. Both groups showed zero percent of similarity with one other hence showed 100% of genetic diversity. Group A was further classified into 2 sub-groups A-1 and A-2 and 5% genetic similarity was observed in them. The sub-groups A-1 and A-2 were additionally divided into clusters A-1-1, A-1-2, respectively and 45% genetic similarity was observed among them (Table 5). B was again sub-divided in groups 1 and B-2 and genetic similarity was 24% between them. Sub-group B-1 was classified into clusters B-1-1 and B-1-2 and it was observed that 28% of similarity was found among them. Out of 42 genotypes, total percentage of genotypes present in cluster A-1-1, A-1-2, A-2-1, A-2-2, B-1-1, B-1-2 holds 21.4 %, 9.5, 7.1, 2.3, 45.2 and 11.9, respectively while sub-group B-2 not further divided into clusters and has only 1 genotype (2.3%). (Table 4.9). PCA analysis was also performed on molecular data to distinguish the genotypes of *linum utisassimum*.

The results of PCA showed that genotypes of both regions (Pak/USA) were grouped into same area which showed that they have some common origin (Fig. 5). The genotype 21570 which is from Pakistan holds the different position in the PCA analysis.

Table. 5. Groups' distribution by cluster analysis for all accessions on the basis of ISSR markers.

Groups	Sub-groups	Clusters	Genotypes	Total %age of all accessions
A	A-1	A-1-1	21750, 31542,20887, 20890,20889, 28359, 31539, 31540, 35756	21.4%
		A-1-2	20888, 31538, 20900, 35011	9.5%
	A-2	A-2-1	22305, 31541, 35881	7.1%
		A-2-2	21400	2.3%
B	B-1	B-1-1	20885, 21310,20891, 21160, 35126, 21200, 21245, 21890, 20886, 31951, 35880, 21425, 35031, 35147, 35032, 35037, 35146, 35016, 35127	45.2%
		B-1-2	20893, 21920, 20870, 21195, 35151	11.9%
	B-2		21570	2.3%

Table 4. Group's distribution by cluster analysis for all accessions on the basis of SSR primers.

Group	Sub-group	Cluster	Genotypes	Total
A	A-1	A-1-1	21750, 20886, 20889, 20890, 22305, 20870, 21195, 21200, 31951, 31540, 21570, 21245, 21310, 21425, 35756, 35880, 35126, 35031, 35016	45.2%
		A-1-2	20893, 31542, 21890	7.1%
	A-2	A-2-1	20891, 20900	4.7%
		A-2-2	31538	2.3%
B	B-1	B-1-1	20888, 20887, 31541, 21400, 35147, 35032, 35037, 35146, 35127	21.4%
		B-1-2	21920	2.3%
	B-2	B-2-1	20885, 35881	4.7%
		B-2-2	28359, 21160	4.7%
		B-2-3	31539, 35151, 35011	7.1%

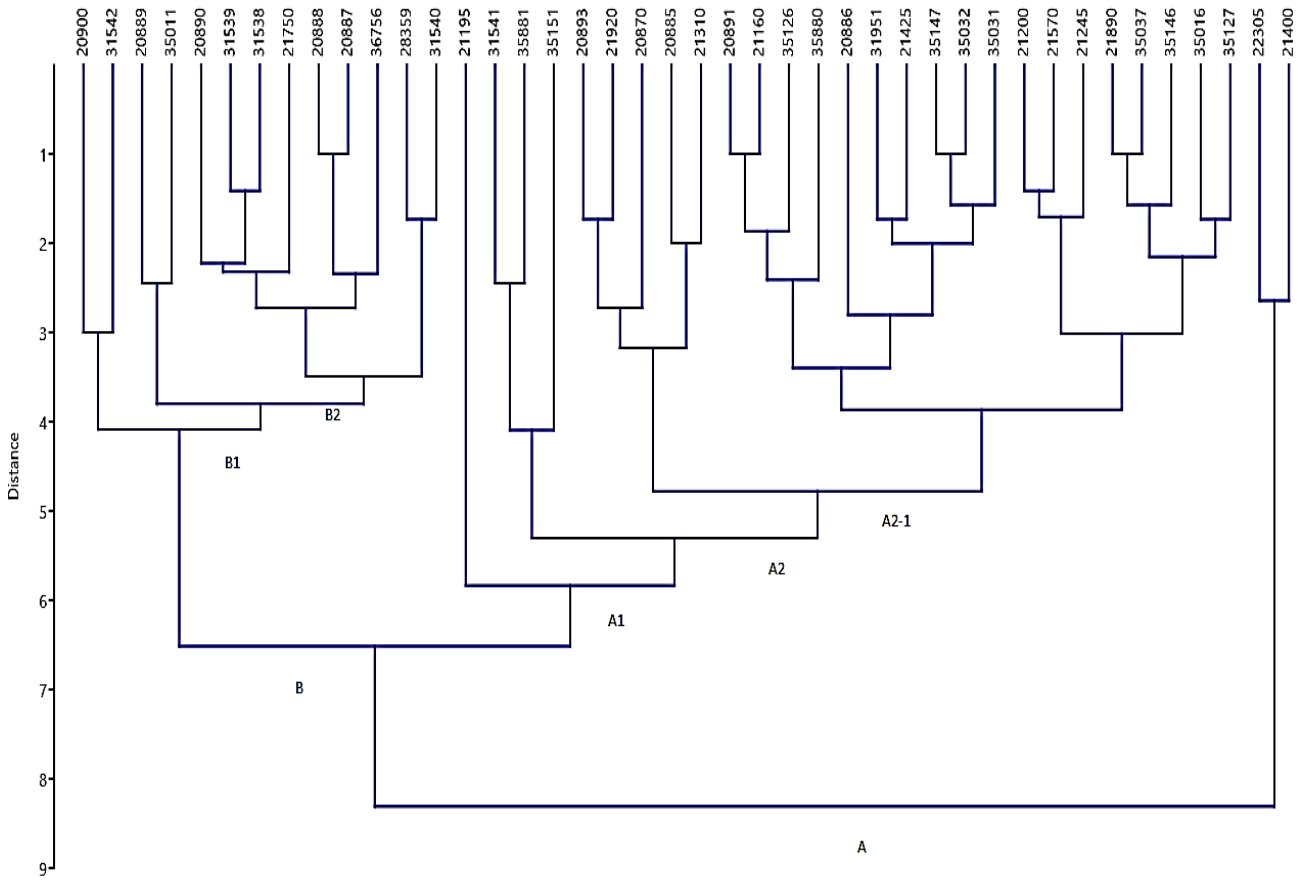

Fig. 3. Group's distribution dendrogram for cluster analysis of linseed accessions on the basis of SSR markers.

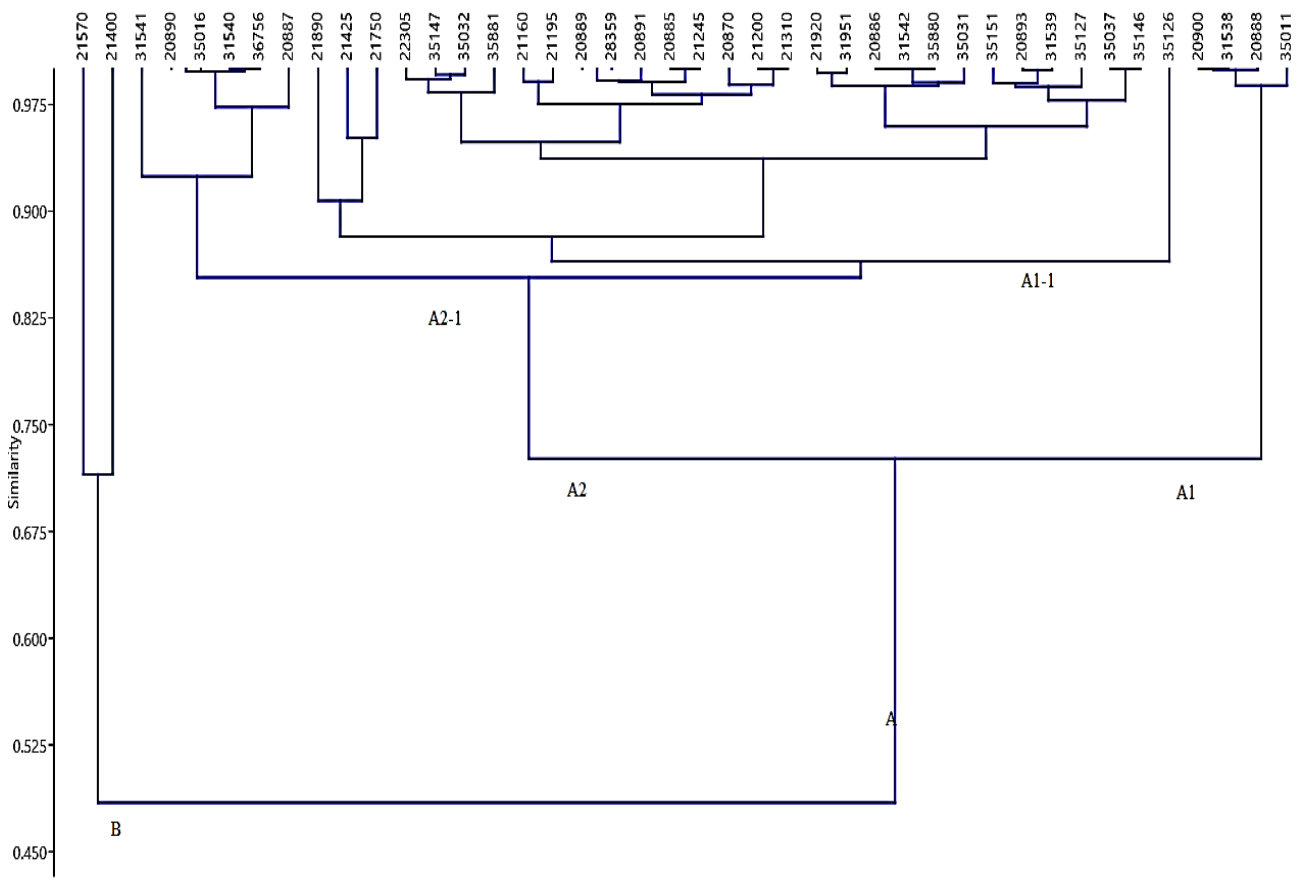

Fig. 4. Group's distribution dendrogram for cluster analysis of linseed accessions on the basis of ISSR markers.

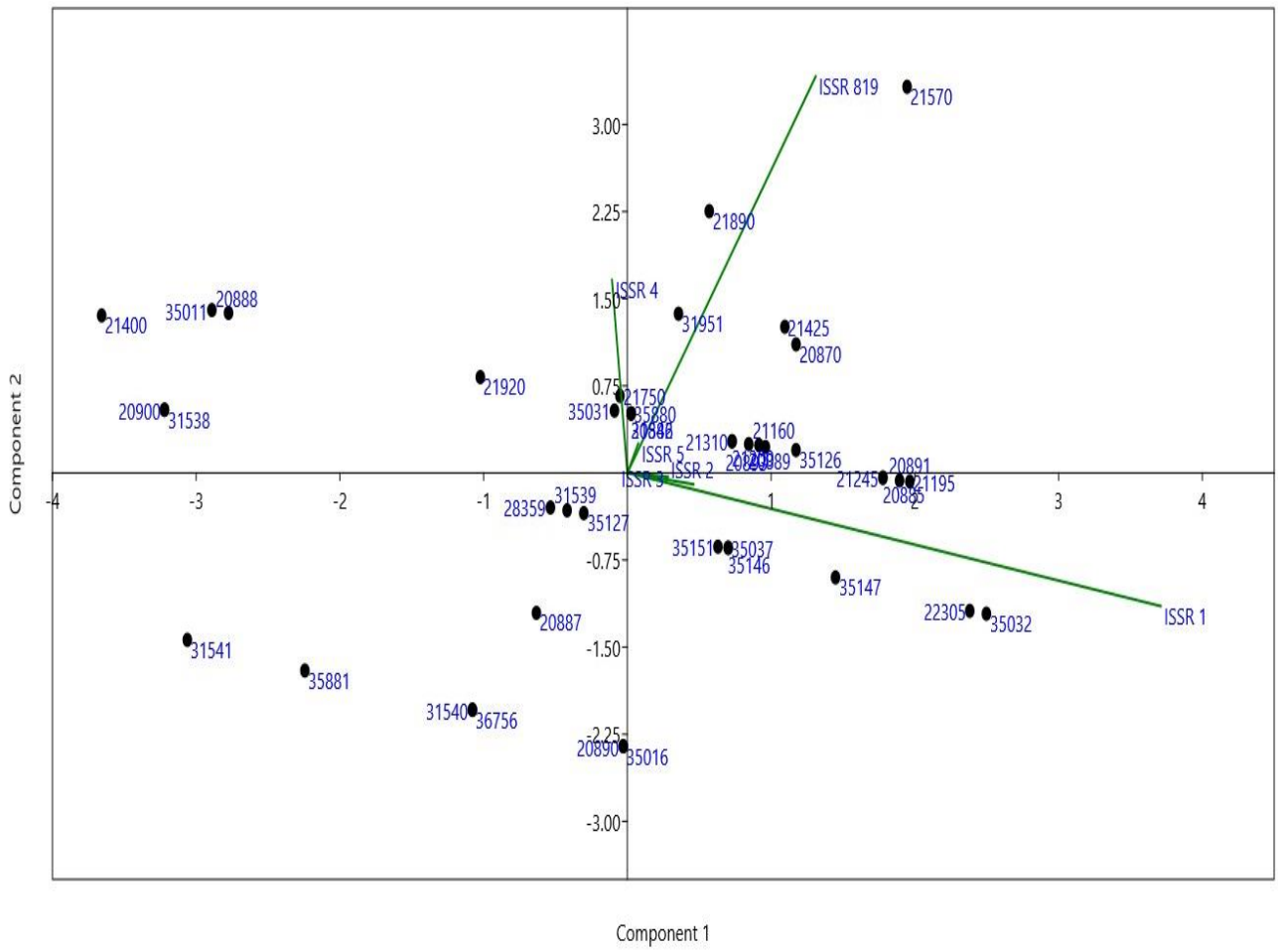

Fig. 5. Biplot analysis of *linum utisassimum* genotypes based on SSR and ISSR data.

Comparison of ssr and issr markers for linseed genotypes: Present research work showed that SSR markers showed valuable information but ISSR were also highly informative as data recorded for 42 lineseed genotypes genetic diversity. Compared to ISSR primers, SSR primers were monomorphic and were easy to record and interpret whereas ISSR primers were polymorphic and showed more polymorphic bands as well as diversity among genotypes. According to many reports, high level of genetic divergence was created by SSR markers as compared to ISSR markers (Mehes, Nkongolo, and Michael, 2007 and 2009; Hamza, Abederrahim, Elbekkay, and Ferchichi 2013 and Kumar and Sharma, 2011). But this work depicted that maximum degree of genetic diversity in genotypes through ISSR primers as compared to SSR primers and ISSR markers were polymorphic and generated many inter-clusters in dendrogram.

Discussion

To understand the genetic range in linseed is indispensable for applicable breeding strategies which can be used to develop the varieties which will fulfill the high domestic and trade demand. Genetic expansion showed a regular, efficient and continuous process which is important step to search for new variations which are the source for advance genetic progress. The present study was carried out to characterize the 42 linseed (*Linum usitatissimum* L.) genotypes through molecular markers. The crop is cultivated for its multiple uses like nutritional, medicinal and industrial importance. It is found to be difficult to explain the purpose of cultivation of *Linum usitatissimum* L., either for its seeds or for its fibers (Kislev *et al*., 2011). The morpho-genetic diversity of genotypes was identified to develop new and improved varieties for future use. The diversity based on morphological traits usually varies with the environment and it is essential to grow the plant to full maturation, however the molecular techniques help to identify great amount of loci in the genome (Jonah *et al*., 2011). For linseed, diverse classes of genetic markers were applied for the evaluation of mapping and range (Clotier *et. al*., 2010; Spilmeyer *et al*., 1998). The SSR markers were used mostly (Chang *et al*., 2015; Caine *et al*., 2014; Cuei *et al*., 2013) to assess the diversity of linseed. It is evident that present study is useful in different ways. The grouping of the genotypes based on morphological data revealed significant data to consider more on genetic diversity on Pakistani and American germplasm and this will be helpful in selecting the suitable genotype with diverse background. This research was based on the use of SSR markers distinguish genotypes on basis of their gene association through cluster analysis and were clustered into nine clusters. The scoring of bands data was based on bivariate data matrix. Similar calculations were carried out by Abou El-Nasr and Mahfouze, 2013, for the

assessment of genetic diversity among linseed genotypes bivariate data matrix was used to conclude the presence and absence of bands. In this work out of five SSR markers 4 were monomorphic except one, LU 24. Our findings are not in accrdance with the result of Singh, Mehta & Sao (2015) who reported the polymorphic nature of SSR markers (LU 1, LU 7, LU 9, LU 8, LU 24 and LU 25) which were used in assessing genetic purity among linseed genotype. Same contradiction were also obtained by Pali *et al*., 2015, who concluded LU 25 to generate polymorphic bands. Screening and selection of linseed genotypes was necessary to enhance genetic pool of species and it is achieved through appropriate study of genetic diversity in individuals. ISSR markers effectively analyze genetic diversity in numerous crop plants (Gautam *et al*., 2016). ISSR markers are reliable, easy and fast to use as well as they produced multi-locus pattern with high polymorphism (Nkongolo *et al*., 2014). A total of 1296 bands were produced by using ISSR markers. The maximum bands (46) and minimum (15) bands through ISSR were obtained by genotype 21570 and 20900, respectively. Thus the more diverse genotype assessed by ISSR marker was 21750. Our results were in accordance by Iqbal *et al*., 2013, who reported that in his study two linseed genotypes showed differences with other genotypes regarding their tendency of reproducible bands V-28 produced maximum (77) whereas V-36 contributed minimum bands (45). Cluster analysis showed that 42 genotypes of linseed were grouped into six main clusters of which the genotype 21400 was found to be more diverse as compared to other genotypes while genotype 21570 does not group in any cluster. Similar reports were obtained by Pali *et al*., 2015 who reported the cluster analysis of 48 genotypes of *Linum usitatissimum* L. by UPGMA method to categorize these genotypes into two main clusters. Two genotypes were found to be more diverse as compared to others hence remained separate. Genetic diversity of Egyptian linseed genotypes were assessed by ISSR primers and primers GAC $(GATA)_4$ and $(GATA)_4$ GC were found to be more informative primer while $(AC)_8T$ and $(GT)_8G$ were less informative in the evaluation of genetic diversity (El Sayed *et al*., 2018). In current study the PIC value of ISSR markers were ranged from 0.48 to 0.975. PIC value showed discerning capability of marker which depends upon number of detected alleles and at their frequency distribution (Chesnokov & Artemyeva, 2015). Characterization of germplasm at molecular level is significant for the sustainable use of crops. ISSR markers being used for genetic diversity evaluation among 203 Ethopian linseed (landraces and varieties) wild Linum species and maximum genetic diversity and PIC values were 0.19 and 0.16 (Mhiret & Heslop-Harrison, 2018).

Conclusion

The genetic diversity was studied by using ISSR and SSR markers based on DNA-PCR technique. SSR markers were monomorphic except LU 24. The useful SSR markers that assessed maximum genetic diversity were LU 10 and LU 24. The genotypes 21750 (Karachi), 21570 (T.T. Singh), 21245 (Jhang), 21425 (Attock) and 21310 (Jhang) showed maximum genetic diversity by SSR markers. The maximum numbers of bands were produced by genotype 21570 with ISSRs PCR product. ISSR markers revealed maximum level of genetic diversity in linseed genotypes as compare to SSR markers.

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