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Research Article

Genetic diversity assessment of flax (*Linum usitatissimum* L.) germplasm using molecular and morphological markers

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Abstract
The present investigation was undertaken to assess the genetic diversity of 48 flax germplasm using molecular and morphological markers. 90 SSR and 10 ISSR flax markers were employed for molecular diversity analysis. Out of these, 25 microsatellite markers were found to be polymorphic giving a total of 99 bands with an average of 3.96 bands per marker ranging from 2 to 12. The polymorphic information content (PIC) value of each SSR primer pair ranged from 0.21 to 0.88 with an average of 0.49 while, in case of ISSR primers, out of 10 primers, only 2 were found polymorphic producing a total of 20 bands with an average of 10 bands per marker ranging from 2 to 11. The polymorphic information content (PIC) value of ISSR primer pair ranged from 0.62 to 0.75 with an average of 0.68. Cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped the 48 flax genotypes into two clusters and ranged from 0.56 to 0.97 with an average similarity index of 0.76. Whereas, in morphological divergence, 48 flax genotypes were grouped into five clusters using Tocher's method in Mahalanobis D^2 statistics. Cluster IV comprising most of the divergent genotypes. The genotypes in cluster III and cluster V exhibited high degree of genetic diversity. Cluster III reflected high cluster means for seed yield per plant, number of capsules per plant, number of primary branches per plant and number of secondary branches per plant. It was evident from both the diversity analysis that two introduced genotypes Neela and R-4158 were the most divergent genotypes followed by PKDL -43, RLC-137, KL-168 and R-4152.

Key words
Clusters, Genetic diversity, DNA, Flax, *Linum usitatissimum*, Molecular markers

Introduction

Flax (*Linum usitatissimum* L.), also called common flax or linseed, is an annual herb, which is the third largest natural fiber crop and one of the five major oil crops in the world (Deng *et al.*, 2011). Flax has been cultivated for several thousand years mainly for its seed oil and its high quality stem fibers. Flax is grown primarily as an oilseed crop used for food and feed as well as in bio-product applications such as linoleum flooring, paint and varnishes. Most oilseed flax varieties are rich in omega-3 (alpha linolenic acid, 55–57%) fatty acid which has been functionally associated with numerous positive health claims.

Diversity analysis of flax is an important component for efficient management and utilization of its genetic resources, and for proper handling of the seed certification programs (Frankel, 1989). 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. That is why breeders look for suitable descriptors or genetic markers. Molecular characterization of flax germplasm has been made using various molecular techniques to assess genetic diversity of cultivated flax and to examine evolutionary relationships of wild flax species (Cloutier *et al.*, 2009; Everaert *et al.*, 2001; Fu and Allaby, 2010 and Rajwade *et al.*, 2010).

Yield components are the primary objectives under study for crop improvement as because Grafius (1978) suggested that there may not be genes for yield *per se* but rather for the various components, the multiplicative interactions of which result in the artifact of yield. In any program aimed at genetic amelioration of yield, genetic diversity is the basic requirement. Effective hybridization program between genetically diverse parents will lead to considerable amount of heterotic response in F_1 hybrids and broad spectrum of variability in segregating generations. Mahalanobis's D^2 statistics is a powerful tool in quantifying the degree of variability at the genotype level. The utility of multivariate analysis have greatly been emphasized (Murty and Arunachalam, 1966).

Present study is a step to select diverse genotypes for flax breeding programme, which can be directly used as a variety or as a parent in crossing programme to develop hybrids. The main objective of this study is to assess the genetic diversity of flax germplasm using DNA-based molecular markers and morphological markers.

Materials and methods

The present study was carried out at Plant Molecular Biology Laboratory and Research cum Instructional Farm, Department of Genetics and Plant Breeding, IGKV, Raipur, India during *Rabi* 2012-2013 in a Randomized Block Design with three replications. The plant materials for this study comprised of 48 flax germplasm (Table 1)

collected from All India Coordinated Research Project (AICRP) on Linseed, IGKV, Raipur, Chhattisgarh, India. Data were recorded on five randomly tagged plants for days to 50 per cent flowering, days to maturity, plant height, number of primary branches per plant, number of secondary branches per plant, number of capsules per plant, number of seeds per capsule, 1000-seed weight, oil content and seed yield per plant. Wilks (1932) criteria were used to test the significance differences in mean values of all the ten characters. Statistical analysis: Mahalanobis (1936) defined the distance between two populations as D^2, which was obtained by Tochers method, described by Rao (1952). Contribution of individual characters towards divergence was estimated according to the method described by Singh and Choudhary (1985). Grouping of variety into various clusters was done and average intra and inter cluster distance were estimated. Analysis of variance was performed to test the significance of difference among the genotypes for the characters studied, as suggested by Panse and Sukhatme (1957). Genetic variability parameters were estimated by the method proposed by Johnson *et al.* (1955).

Genomic DNA Extraction: Genomic DNA was extracted from young leaves of the flax plants according to the modified CTAB method (Kang *et al.,* 1998). The concentrations and quality of the genomic DNA samples were estimated on spectrophotometer ND-2000 (Nanodrop, USA). Finally, all the genomic DNA samples were diluted to a final concentration of 40 ng/µl with TE buffer (10 mM Tris-HC1, pH 8.0; 1 m M EDTA) and stored at -20^0C for further use.

PCR Amplification of SSR and ISSR Markers: A total of 90 flax SSR primers and 10 ISSR primers covering all the chromosomes of flax were used in this study. Polymerase chain reaction for amplifications of DNA preparations were carried out in 20 µl for SSR and 25 µl volume for ISSR (Table 2, 3a and 3b). All PCR reactions were carried out in a Veriti Thermal Cycler (Applied Biosystems, USA). PCR products were separated using 5% PAGE, stained with ethidium bromide and photographed under UV light using Image Gel Doc Lab_{TM} software Version 2.0.1 (Bio-Rad, USA).

Data Analysis: The band profiles were scored only for distinct, reproducible bands as present (1) or absent (0) for each SSR and ISSR primer pair. Jaccard's similarity coefficient values were calculated and dendrogram based on similarity coefficient values were generated using unweighted pair-group method with arithmetic means (UPGMA) by the NTSYSpc 2.10e software (Rohlf, 2000). The polymorphism information content (PIC) value of SSR and ISSR markers was calculated using the following formula by Anderson *et al.* (1993).

$$PIC = 1 - \sum_{i=1}^{k} p_i^2$$

where k is the total number of alleles (bands) detected for one SSR locus and P_i is the frequency of the i^{th} allele (band) in all the samples analyzed.

Results and discussion

SSR and ISSR Amplification: Out of 90 flax SSR primers screened, 25 were found polymorphic revealing 99 bands in 48 genotypes, of which 95 bands were found to be polymorphic in nature with 94.6% polymorphism. All the primers gave 100% polymorphism except LU-19, LU-34 and LU-38 which gave 75 and 82% polymorphism. The PIC value of SSR primers ranged from 0.21 to 0.88. The number of bands per primer varied widely among these primers, ranging from 2 (LU-22) to 12 (LU-8). The average number of bands per primers was 3.96. PIC value was ranged from 0.21 for primer LU-19 to 0.88 for primer LU-8 with an average of 0.49 for all the primers. Details of SSRs markers are given in the Table 4 and the gel photograph of SSR primer LU-6 in Figure 1. Out of 10 ISSR primers, only 2 primers were found polymorphic to differentiate the genotypes. A total of 20 bands were detected using 2 ISSR primers out of which 18 bands were polymorphic and only 2 were monomorphic. The number of amplified bands varied from one in primer UBC-884 to a maximum of 11 in primer UBC-807 with an average of 10 bands per primer. The PIC values ranged from 0.62 for primer UBC-841 to as high as 0.75 for primer UBC-807 with an average of 0.68 for the two ISSR primers. Details of ISSR markers are given in the table 4 and the gel photograph of ISSR primer UBC-807 in Figure 2.

Rooseamsaleg *et al.* (2006) reported that 28 genomic SSR markers exhibited an average of 3.32 when assessed on 93 flax cultivars, while the average alleles per locus of EST-SSR markers was only 2.3 based on 248 EST-SSRs assessed on 23 flax genotypes (Cloutier *et al.,* 2009). These may indicate that genomic SSR markers had higher alleles per locus than EST-SSR markers. Huang and Sun, 2000 observed 2071 ISSR loci with 15 primers in 40 genotypes of Ipomea and 62 per cent of the loci were polymorphic.

The consideration of estimated genetic distance is important for comparative analysis of diversity levels (Roldan-Ruiz *et al.,* 2001). SSR and ISSR analysis is a powerful tool to discriminate and cluster closely related cultivars as well as to trace origin and pedigree through genepool sharing. SSR and ISSR analysis covers the entire genome, compared to morphological analyses that focus on a few traits.

k

Cluster Analysis: Cluster analysis was carried out based on the similarity index data derived from the SSR and ISSR markers which grouped the 48 genotypes into two major clusters (Figure 3). The Jaccard's similarity ranged from 0.56 to 0.97 with an average similarity index of 0.76 indicating that most of the varieties used in the present study were having common ancestors in their pedigree. The genotype Polf-22 showed the highest similarity with LCK-88068 (*i.e.* 0.965) while Neela showed the least similarity with R-4158 (*i.e.* 0.688). High level of similarity was also found among GS-129 and RLC-122 (0.965) as well as between Meera and PKDL-62 (0.930). Cluster analysis based on genetic similarity values provided a clear resolution of relationships among all the 48 flax genotypes. Two major clusters were observed at 0.57 of genetic similarity coefficient index (Figure 3). First major cluster contained 38 flax genotypes with an average similarity index of 0.603. Further, it was sub-grouped into two clusters, IA and IB having 24 and 14 genotypes, respectively. The similarity coefficients between any two flax genotypes in the sub-cluster, IA ranged from 0.648 (FRW-12 and R-4168) to 0.965 (POLF-22 and LCK-88068) with an average of 0.810. Second, sub-cluster, IB was more diverse than first sub-cluster, IA. The similarity coefficients of sub cluster, IB between any two genotypes varied from 0.613 (Shekhar and Deepika) to 0.930 (Meera and PKDL-62) with an average of 0.785. The second major cluster included 10 flax genotypes with similarity indices from 0.681 (RLC-133 and Neela) to 0.908 (RLC-135 and R-4141) with an average of 0.780.

The highest genetic distances were mostly obtained from pairs of exotic and local collections, indicating their wide dissimilarity. Most of the clusters were not observed to contain all genotypes assembled from a particular collection area, indicating the absence of correspondence between the genotypes and their geographical origin. It was evident from the cluster (Figure 3) that two introduced genotypes (Neela and R-4158) were most distantly related to the remaining genotypes followed by PKDL-43, RLC-137, KL-168 and R-4152. This shows that range of genetic diversity was greatest among genotypes contained in these two major clusters and cross hybridising between them may increase genetic variation in the breeding population. Similar results were also reported in the diversity studies of maize inbred lines and perennial ryegrass (Roldan-Ruiz *et al.*, 2001).

The knowledge of genetic variation is useful to select parents for breeding programs. Several researchers have reported the use of molecular genetic distance to select diverse parents, which when crossed, gave higher heterosis in various crop plants (Riaz *et al.,* 2001; Joshi *et al.,* 2001). The varieties analyzed in this study, *viz*., 'Neela', 'R-4158' and PKDL-43, are agronomically superior and genetically the most distant from other genotypes, as revealed by SSR and ISSR-PCR analysis. Their potential for containing unique alleles can be exploited by crossing them with other elite lines from different genetic clusters.

Mahalanobis D^2 Analysis: Success of any crop improvement programmes mainly depends on amount of diversity available in the crop. The genotypes belonging to diverse ecological regions may clustered together, while those of same region may entered into separate groups. In the present investigation, 48 genotypes were grouped into five clusters on the basis of D^2 statistics (Table 5). Cluster IV was the biggest with 15 genotypes followed by cluster III and V with 9 genotypes each, cluster II contains 8 genotypes, while, the lowest entries were found in the cluster I contains 7 genotypes (Table 5). The average D^2 values ranged from 2.314 to 5.847. Intra cluster distances exhibited a range of 2.314 to 3.039 and inter cluster distance ranged from 2.977 to 5.847 (Table 6). From the present investigation, it was clear that cluster IV comprising the most divergent cluster. Cluster III showed maximum inter cluster distance with cluster V. Therefore, it can be concluded that the genotypes present in these clusters can be utilized for successful hybridization programme.

Contribution of each character towards genetic divergence has been estimated from the number of times that each character appeared in the first rank (Table 7). It has been observed that 50 per cent flowering was highest contributor towards divergence followed by oil content, plant height, days to maturity, seed yield per plant and plant height. While, number of secondary branches per plant was the least contributor towards total divergence. Therefore, these characters should be given importance during hybridization and selection of segregating populations. Similar results were also obtained by Begum *et al.* (2007) and Tadesse *et al.* (2009). Further, analysis of cluster means indicates diversity demonstrated by different clusters for a character. Based on the means, it is possible to know the character influencing divergence. The variation observed in cluster mean also points to the degree of variability (Verma, 1996 and Verma, 1999). The cluster means from various characters are presented in (Table 7). The cluster III had the highest mean values for number of primary branches per plant (8.56), number of secondary branches per plant (27.22), number of capsules per plant (168.96), seed yield per plant (13.82), and genotypes in cluster I showed highest mean values for plant height (63.38), number of seeds per capsule (8.36), oil content (40.22). Similarly, genotypes in cluster V had highest mean values for days to maturity

(119.37), 1000 seed weight (7.06), whereas the cluster IV recorded highest mean values for days to 50 per cent flowering (61.13). The genotypes included in these clusters may be utilized in future breeding programme to enhance the yield and its components.

Multivariate analysis based on D^2 analysis and canonical varietal analysis has been considered as an important tool for quantifying genetic divergence in different crops (Rao, 1952). This analysis also provides a measurement of relative contribution of different components on diversity both at intra and inter-cluster level (Murthy and Quadri, 1996). The 48 germplasm of flax subjected to D^2 analysis for ten quantitative characters. Based on D^2 values five clusters were formed. Most of the genotypes derived from different crosses were grouped in a single cluster (cluster V), indicating the similarity for the character studied in parental material. On contrary, the genotypes having similar pedigree were grouped into different clusters. It clearly demonstrated the impact of selection in increasing the genetic diversity. These results are in conformity with the findings of Laxminarayana *et al.* (2004). The intra-cluster distance ranged from 3.039 to 2.314. It indicated wide range of diversity in terms of genetic distance (D^2 value). Thus it was evident that considerable amount of genetic divergence was present in the material under study. Maximum inter-cluster distance was observed between cluster III and V (5.847) followed by cluster II and III (5.720) indicating maximum diversity between the genotypes of these clusters with respect to the traits considered. However, the lowest interaction distance was observed between cluster II and V (2.977) inferring the similarity for most of the characters among the genotypes of the respective clusters. Based on inter-cluster distance and cluster means, it can be revealed that genotypes of the cluster III and V could be selected for hybridization programmes as they are expected to produce high heterotic crosses. The most diverse clusters among all clusters were cluster IV. Results indicated that inter crossing of genotypes from different clusters showing superior mean performance may help in obtaining high yield. The genotypes from above different clusters may be utilized as parents in crossing programme to isolate desirable segregants for yield traits. Selection of parents need not be necessary based on geographical diversity, the genetic diversity may prove more sound base for the purpose.

Conclusion

In this study, we confirmed that SSR and ISSR markers, as a fast and simple technique, can detect enough polymorphism to differentiate flax germplasm and to understand their inter-relationships. These markers can potentially be used in flax for germplasm identification, genetic diversity study, and particularly in genetic mapping and breeding for marker assisted selection. From both the diversity evaluation, it is advocated that the genotypes namely Neela, R-4158 followed by PKDL-43, RLC-137, KL-168 and R-4152 were identified as the most divergent genotypes for seed yield per plant. The cluster III (PKDL-62, GS-129, RLC-134, R-552, CI-229, NL-97, FRW-12, Gcwargi 1-2, R-4129) and cluster V (Kiran, RLC-92, Meera, RLC-94, RLC-132, RLC-133, R-4140, R-4141, R-4154) was most diverse to each other. Hence, crossing between these clusters would help to accumulate favorable and desirable alleles for further improvement in seed yield and its component in flax.

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References

Anderson, J.A., Churchill, G.A., Sutrique, J.E., Tanksley, S.D. and Sorrells, M.E. 1993. Optimizing parental selection for genetic linkage maps. *Genome*, **36**: 181-186.

Begum, H., Alam, A.K.M.M., Chowdhary, M.J.A. and Hossain, M.I. 2007.Genetic divergence in linseed (*Linum usitatissimum* L.). *Int. J. Sustain. Crop Prod*., **2**(1): 04-06.

Cloutier, S., Niu, Z., Datla, R. and Duguid, S. 2009. Development and analysis of EST-SSRs for flax (*Linum usitatissimum* L.). *Theor. Appl. Genet.*, **119**: 53–63.

Deng, X., Long, S., Dongfeng, H., Xiang, L., Wang, Y.F., Hao, D.M., Qiu, C.S. and Chen, X.B. 2011. Isolation and characterization of polymorphic microsatellite markers from flax. *Afr. J. Biotech.*, **10**(5): 734-739.

Everaert, I., R-Iek, J.D., Loose, M.D., Waes, J.V. and Bockstaele, E.V. 2001. Most similar variety grouping for distinctness evaluation of flax and linseed (*Linum usitatissimum* L.) varieties by means of AFLP and morphological data. *Plant Var. Seeds*, **14**(2): 69-87.

Frankel, O.H. 1989. Practical considerations relevant to effective evaluation. In: Brown A.H.D., Frankel, O.H., Marshall, D.R. and Williams, J.T. (eds), The use of plant genetic resources. Cambridge University Press, Cambridge, pp. 235–260.

Fu, Y.B. and Allaby, R.G. 2010. Phylogenetic network of *Linum* species as revealed by non coding chloroplast DNA

sequences. *Genet. Resour. Crop Evol.*, **57**(5): 667-677.

Grafius, J.E. 1978. Multiple characters and correlated response. *Crop Sci*., **18**: 931-934.

Huang, J.C. and Sun, M. 2000. Genetic diversity and relationships of sweet potato and its wild relative in Ipomoea series Batatas (Convolvlaceae) as revealed by Inter-Simple Sequence Repeat (ISSR) and restriction analysis of chloroplast DNA. *Theor. Appl. Genet.,* **100**: 1050-1060.

Johnson, H.W., Robinson, H.P., Comstoc, R.E. 1955. Estimation of genetic and environmental variability in soybeans. *Agron. J.*, **47**: 314-318.

Kang, H.W., Yong, G.C., Unghan, Y. and Moo, Y.E. 1998. A rapid DNA extraction method for RFLP and PCR analysis from a single dry seed. *Plant Mol. Bio. Rep.,* **16**: 1-9.

Laxminarayana, V.N., Sreedhar, N. and Prabhakaran, A.J. 2004. Genetic divergence analysis in sunflower (*Helianthus annuus* L.). *J. Oilseeds Res.,* **21**(1): 162-164.

Mahalanobis, P.C. 1936. Study on the generalized distance in statistics *Proc. Nat. Ins. Sci.India*, **2**: 49-55.

Murty, B.R. and Arunachalam, V. 1966. The nature of divergence in relation to breeding systems in some crop plants. *Indian J. Genet.,* **26**A: 188-198.

Murthy, V.R. and Quadri, M.L. 1996. Analysis of divergence in some self-compatible forms of *Brassica campestris var.* Brown sarson. *Indian J. Genet.,* **26**: 45-48.

Panse, V.G. and Sukhatme, P.V. 1957. Statistical methods for agricultural workers, ICAR, New Delhi, pp. 100-108.

Rajwade, A.V., Arora, R.S., Kadoo, N.Y., Harsulkar, A.M., Ghorpade, P.B. and Gupta, V.S. 2010. Relatedness of Indian flax genotypes (*Linum usitatissimum* L.): An Inter-Simple Sequence Repeat (ISSR) primer assay. *Mol. Biotech.,* **45**: 161-170.

Rao, C.R. 1952. Advanced statistics methods in biometry, John Wiley, New York, pp. 357-369.

Riaz, A., Li, G., Quresh, Z., Swati, M.S. and Quiros, C.F. 2001. Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence related amplified polymorphism and its relation to hybrid performance. *Plant Breed.,* **120**(5): 411-415.

Rohlf, F.J. 2000. NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.10e. Exeter publications, New York.

Roldan-Ruiz, I., Van-Eeuwijk, F.A., Gilliland, T.J., Dubreuil, P., Dillmann, C., Lallemand, J., De-Lose, M. andBaril, C.P. 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.

Roose-Amsaleg, C., Cariou-Pham, E., Vautrin, D., Tavernier, R. and Solignac, M. 2006. Polymorphic microsatellite loci in *Linum usitatissimum. Mol. Ecol. Notes,* **6**: 796-799.

Singh, R.K. and Choudhary, B.D. 1985. Biometrical methods of quantitative genetic analysis. *Haryana J. Hort. Sci.,* **12**: 151-158.

Tadesse, T., Singh, H. and Weyessa, B. 2009. Genetic divergence in linseed germplasm. *J. Innov. Dev. Strategy,* **3**(2):13-20.

Verma O.P. 1996. Genetic divergence in linseed (*Linum usitatissimum* L.). *J. Oilseeds Res*., **13**(2): 225-228.

Verma, V.D. 1999. Genetic diversity in linseed. *Indian J. Plant Genet. Resour.,* **12**(1): 16-19.

Wilks, S.S. 1932. Certain generalization in the analysis of variance. Biometrics, **24**: 471-494.

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Table 1. Details of the 48 flax germplasm used in the study

S. No	Genotypes	Source	S. No.	Genotypes	Source
1.	Kiran	Raipur, India	25.	GS-64	Gurdaspur, India
2.	Deepika	Raipur, India	26.	GS-129	Gurdaspur, India
3.	Kartika	Raipur, India	27.	RLC-92	Raipur, India
4.	Indira Alsi-32	Raipur, India	28.	RLC-94	Raipur, India
5.	Shekhar	Kanpur, India	29.	RLC-122	Raipur, India
6.	Neela	Berhampore, India	30.	RLC-123	Raipur, India
7.	Rashmi	Kanpur, India	31.	RLC-128	Raipur, India
8.	Sharda	Jhansi, India	32.	RLC-129	Raipur, India
9.	Meera	Kota, India	33.	RLC-132	Raipur, India
10.	PKDL-43	Pawarkheda, India	34.	RLC-133	Raipur, India
11.	PKDL-58	Pawarkheda, India	35.	RLC-134	Raipur, India
12.	PKDL-62	Pawarkheda, India	36.	RLC-135	Raipur, India
13.	JRF-5	Kanpur, India	37.	RLC-137	Raipur, India
14.	JLS-9	Jabalpur, India	38.	R-552	Raipur, India
15.	KL-1	Palampur, India	39.	CI-229	Ludhiana, India
16.	KL-168	Palampur, India	40.	NL-97	Nagpur, India
17.	GS-27	Gurdaspur, India	41.	Polf-22	Poland, Europe
18.	GS-61	Gurdaspur, India	42.	T-397	Kanpur, India
19.	LC-54	Ludhiana, India	43.	R-4140	Raipur, India
20.	LCK-88068	Kanpur, India	44.	R-4141	Raipur, India
21.	FRW-12	France, Europe	45.	R-4152	Raipur, India
22.	Gcwargi 1-2	Kanpur, India	46.	R-4154	Raipur, India
23.	R-2678	Raipur, India	47.	R-4158	Raipur, India
24.	R-4129	Raipur, India	48.	R-4168	Raipur, India

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Table 2. PCR components using SSR and ISSR markers

Components	Microsatellite (SSR) (µl)	ISSR (µl)
Nanopure water	13.5	15.5
10 X PCR Buffer	2	2.5
dNTPs 10mM	1	1.5
Primer (F) 5 pmol	0.5	1.5
Primer (R) 5 pmol	0.5	-
Taq 1U/ µl	0.5	1.0
Template DNA (40 ηg/µl)	2	3
Total volume	20	25

Table 3a. Temperature profile used for PCR amplification for SSR markers

Steps	Temperature (°C)	Duration (min.)	Cycles	Activity
1	95	4	1	Initial Denaturation
2	95	1	32	Denaturation
3	58	1		Annealing
4	72	1		Extension
5	72	7	1	Final Extension
6	4	24 hrs.		Storage

Table 3b. Temperature profile used for PCR amplification for ISSR markers

Steps	Temperature (°C)	Duration (min.)	Cycles	Activity
1	95	4	1	Initial Denaturation
2	95	0:30	35	Denaturation
3	53	1		Annealing
4	72	1:30		Extension
5	72	15	1	Final Extension
6	4	24 hrs.		Storage

Table 4. Characteristics of the 25 SSR and 2 ISSR markers of *Linum usitatissimum*.

S.No	Primers	Sequence 5'----------> 3'	K	$T_a(^0C)$	Allele Size (bp)	PIC Value
		SSR Primers				
1	LU-1	**F:**TCATTCATCTCCTTCCACTAAAA **R:**TTGAAAGCCCTAGTAGACACCA	4	58	146-179	0.630
2	LU-2	**F:**TCCGGACCCTTTCAATATCA **R:**AACTACCGCCGGTGATGA	3	58	139-148	0.468
3	LU-5	**F:**GTCACTGGGTGTGTGTTTGC **R:**AGCAGAAGAAGATGGCGAAA	3	58	134-140	0.371
4	LU-6	**F:**CCCCATTTCTACCATCTCCTT **R:**CAACAGCGGAACTGATGAAA	3	58	125-137	0.468
5	LU-7	**F:**CATCCAACAAAGGGTGGTG **R:**GGAACAAAGGGTAGCCATGA	5	58	134-146	0.595
6	LU-8	**F:**TCCCGTAATATTCTATGTTCTTCC **R:**TGAGTTGGACCTTACAAGACTCA	12	58	144-228	0.881
7	LU-9	**F:**TTGCGTGATTATCTGCTTCG **R:**ATGGCAGGTTCTGCTGTTTC	4	58	102-150	0.605
8	LU-10	**F:**GCCTAAAGCTGATGCGTTTC	5	58	141-159	0.701

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		R:TGTCAGGCTCCTTCTTTTGC				
9	LU-11	**F:**ATGGCAGGTTCTGCTGTTTC	5	58	105-153	0.618
		R:TTGCGTGATTATCTGCTTCG				
10	LU-15	**F:**TGGACGACGATGAAGATGAA	3	58	108-114	0.511
		R:CCGCCGGGTACACTACTACT				
11	LU-17	**F:**GCTGGACCTTACAAGCCTCA	3	58	144-150	0.427
		R:TTGGTGGGAGAACAACAAGA				
12	LU-18	**F:**AGAGGCGGAGGGCATTAC	4	58	139-145	0.443
		R:TTGGAGAGTTGGAATCGAGA				
13	LU-19	**F:**TCTCAGCTCCCTTTTATTTACCC	3	58	132-147	0.215
		R:GCAGTCTCGAGTGCTGAGTG				
14	LU-20	**F:**TTCAACCAGGCAAATTTCAA	2	58	108-123	0.359
		R:CAAGAAGAGGCCCAGAATTG				
15	LU-21	**F:**AAGGGTGGTGGTGGGAAC	4	58	97-147	0.630
		R:GTTGGGGTGAAGAGGAACAA				
16	LU-22	**F:**GATGGGGTTGAAGCCAGTAG	2	58	138-144	0.375
		R:CCCACCCCATCTATCATTTG				
17	LU-24	**F:**ATGGCAGGTTCTGCTGTTTC	8	58	90-138	0.746
		R:TTGCGTGATTATCTGCTTCG				
18	LU-25	**F:**TCTACAGAGTTCAATTCCCGTAA	6	58	153-204	0.754
		R:GTTGGACCTTACAAGACTCACTG				
19	LU-27	**F:**GTTTGAGAAGAGGGCATCCA	4	58	158-167	0.387
		R:GTTGGGGTGAAGAGGAACAA				
20	LU-29	**F:**GGGCAGTGATTGATTGGTTT	2	58	118-124	0.305
		R:GGCGGCAATTGCTACATT				
21	LU-31	**F:**TCTTTGTTTGGTGCCAAAGTT	4	58	97-112	0.510
		R:TTCATGATCTCACCTAACCTGA				
22	LU-32	**F:**ACGCGTAAACTTTCCGTTTC	3	58	144-150	0.511
		R: ATAATGTCGGCTGCTTCTGC				
23	LU-33	**F:**TTCTCCATCATCTCACATCCA	3	58	150-174	0.398
		R: CCAAATCAGAATGTGCGTGT				
24	LU-34	**F:**GGAAGAATTGGAAGAGGAAGG	2	58	128-134	0.258
		R:CCTTCTCCCATGATCAAACAA				
25	LU-38	**F:**GATCTTGTTGCCTGGGAAAG	2	58	80-104	0.258
		R:TTCGTTTGCAATACGTCAGC				
		ISSR Primers				
1	UBC-807	AGAGAGAGAGAGAGAGT	11	53	200-800	0.750
2	UBC-841	GAGAGAGAGAGAGAGACC	9	53	200-1000	0.625

K: number of allele; T_a: Annealing temperature; PIC: polymorphic information content

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Table 5. Distribution of 48 flax germplasm into different clusters

Clusters No.	No. of genotypes	Name of the genotypes
I	7	Deepika, Kartika, Indira Alsi-32, Shekhar, T-397, RLC-122, Polf-22
II	8	Rashmi, Sharda, JLS-9, GS-27, GS-61, GS-64, R-2678, LCK-88068
III	9	PKDL-62, GS-129, RLC-134, R-552, CI-229, NL-97, FRW-12, Gcwargi 1-2, R-4129
IV	15	Neela, PKDL-58, PKDL-43, JRF-5, KL-1, KL-168, RLC-123, RLC-128, RLC-129, RLC-135, RLC-137, LC-54, R-4152, R-4158, R-4168
V	9	Kiran, RLC-92, Meera, RLC-94, RLC-132, RLC-133, R-4140, R-4141, R-4154,

Table 6. Average intra and inter-cluster D^2 value of 48 flax germplasm

Clusters	I	II	III	IV	V
I	**2.476**	3.654	3.848	3.289	3.280
II		**2.432**	5.720	3.429	2.977
III			**3.021**	3.964	5.847
IV				**3.039**	3.401
V					**2.314**

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Table 7. Cluster means and percent contribution for yield and its components in 48 flax germplasm

Clusters	I	II	III	IV	V	Contribution (%)
Days to 50 % flowering	52.14	51.08	58.89	61.13	49.04	41.22
Days to maturity	113.83	114.17	115.89	117.22	119.37	13.21
Plant height	63.38	49.42	61.48	59.11	60.0	2.48
Number of primary branches per plant	5.71	4.83	8.56	6.20	3.30	1.24
Number of secondary branches per plant	16.19	13.54	27.22	17.67	12.04	0.09
Number of capsules per plant	92.33	64.88	168.96	86.64	70.81	0.00
Number of seeds per capsule	8.36	6.85	8.07	7.80	8.23	0.71
1000 seed weight	6.10	6.50	5.91	5.80	7.06	1.6
Oil content	40.22	38.55	37.76	37.66	38.71	36.7
Seed yield per plant	11.10	6.03	13.82	7.13	5.91	2.75