Electronic Journal of Plant Breeding, 6(3): 848-854 (Sep 2015)
ISSN 0975-928X

Research Note

Genetic diversity in indigenous and exotic linseed germplasm (*Linum usitatissimum* L.)

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(Received:13 Jan 2015; Accepted:28 May 2015)

Abstract
Field experiments were conducted involving 103 indigenous and exotic accessions of linseed germplasm during *Rabi* 2011-12 and *Rabi* 2012-13 at ICAR-NBPGR Regional Station, Akola to assess the extent of genetic diversity in the germplasm based on seven quantitative traits. Using Mahalonobis' D^2 distance, the germplasm were grouped into 12 clusters. Cluster I was the largest with 23 accessions, cluster XII was smaller with two accessions and clusters V, VI, VIII, X and XI were the smallest with one accession in each. The contribution of seed oil content and 1000 seed weight towards genetic divergence were higher. The inter-cluster distance was the farthest between cluster IX and cluster XII and cluster VIII and XII. Hybridization between IC 567363 in cluster VIII *versus* EC 541215 and EC 541218 in cluster XII and EC 541198, IC 397953, IC 267673 in cluster IX *versus* EC 541215 and EC 541218 in cluster XII is suggested to obtain desirable progenies.

Keywords
Linum usitatissimum, germplasm, diversity, Mahalanobis D square

Linseed is believed to be originated in the region east of the Mediterranean Sea (Simmonds, 1976; Zohary 1999). It is also believed that domestication of fibre flax might have occurred in India and China whereas, seed type linseed occurred in South Eastern Asia. The number of linseed germplasm collected from all over the World is estimated to be around 55,000 to 60,000 (Diederichsen, 2007). Presently, linseed germplasm collected from different countries are conserved in 38 research centres and gene banks spreading in 28 countries (Diederichsen and Richards, 2003; Diederichsen, 2007). The Indian gene banks holds 5,095 accessions at ICAR-National Bureau of Plant Genetic Resources, New Delhi and All India Coordinated Research Project on Linseed, Kanpur (Radhamani *et al*., 2006). The NBPGR Regional Station, Akola, Maharashtra is maintaining 722 accessions of indigenous and exotic origin. Genetic diversity is the pattern of variation, combination of different genes found within the population of a single species. Inclusion of genetically divergent parental lines in the hybridization programme will enable the breeders to combine desirable genes in new recombination or to obtain heterotic crosses. Understanding the importance of genetic diversity in linseed improvement, a part of the germplasm accessions (103 accessions) maintained at Akola Centre was evaluated for economically important traits for two years.

The germplasm for the study consisted of 75 indigenous accessions of seed flax (*Linum usitatissimum*) collected from Bihar, Himachal Pradesh, Jharkhand, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Uttar Pradesh, Uttarakhand and West Bengal and 25 exotic accessions, obtained through NBPGR, from Argentina, Australia, Russia and USA and three check varieties H-local, Garima and Kiran. Field experiments were conducted under irrigated condition in black soil during *rabi* 2011-12 and *rabi* 2012-13 at ICAR-NBPGR Regional Station, Akola (Maharashtra) in RBD with two replications. Each accession was sown in three meter long rows. The spacing adopted was 60 x 10 cm. The package of practices as recommended in the Maharashtra State for linseed was followed to raise good crop. Observations on seven quantitative traits *viz.*, days to 50% flowering, days to maturity, plant height, number of capsules per plant, 1000 seed weight, seed yield per plant and oil content were recorded. The pooled mean of two years was utilized for estimating the variances and covariances as per the procedures given by Singh and Choudhary (1977). Genetic divergence and number of cluster was worked out using Mahalanobis D^2 statistics (Mahalanobis, 1936) and Toucher's method described by Rao (1952). The average intra and inter cluster distances were estimated as per the procedure outlined by Singh and Choudhary (1977)

The variances, co-variances and determinants were estimated from the pooled mean 2011-12 and 2012-13 data. Using *V* statistics which utilize Wilk's criteria, a simultaneous test of differences between mean values of number of correlated variables was carried out. The values (V stat=1623;

probability=0) were significant. Hence, it was proceeded to estimate Mahalanobis generalized distance (D^2 values) among 103 genotypes.

1. Contribution of individual characters towards genetic divergence: Of the seven traits, the most important trait contributing towards the divergence in linseed (Table 1) were the number of capsules per plant (28.19%) and days to maturity (23.03%). The contribution of days to 50% flowering (17.67%), plant height (16.98%), seed yield (7.58%), were next in order. Hence, these traits must be recorded invariably while evaluating germplasm. The contribution of 1000 seed weight (3.73%) and seed oil content (2.82%) towards genetic divergence were negligible.

2. Clustering of genotypes: By treating the estimated D^2 values as the square of the generalized distance and by following Tocher's method of clustering, the 103 linseed accessions were grouped into 12 clusters. The number of clusters and their constituent accessions are shown in Table 2. Cluster I was the largest with 23 accessions, cluster XII was smaller with two accessions and Clusters V, VI, VIII, X and XI were the smallest with one accession in each. Such a large and diverse clustering pattern is an indication of existence of significant amount of variability and diversity in the germplasm.

3. Group constellation: Nineteen out of 20 accessions belongs to cluster I were originated in India while EC 001475 alone was from Australia (Table 2). Cluster VII was the second largest in size with 20 accessions, from Argentina (EC 041762), Russia (EC 541203 and EC 541205) and India (17 accessions). Cluster II was the 3rd largest comprising 19 accessions. Three accessions in the cluster namely, EC 399082, EC 399084 and EC 399085 were originated from USA. The remaining 16 accessions were indigenous collections from Himachal Pradesh, Uttarakhand and Uttar Pradesh. The fourth largest cluster was cluster IV. It was composed of 18 accessions, 10 of which (all EC series accessions in that cluster) were from Russia, two from Bihar (IC 249009 and IC 249012), one each Madhya Pradesh (IC 268337), West Bengal (IC 212041) and 2 accessions each from Maharashtra (IC 118890, IC 054951) and Uttar Pradesh (IC 356292, IC 356126). Cluster III was composed of 9 indigenous accessions and one (EC 541206) exotic accession. Cluster IX was composed of 6 accessions, three of which were Russian origin and another three accessions were of India origin. Cluster XII comprised of two accessions namely EC 541211 and EC 541214. Both were originated in Russia. Cluster number V, VI, VIII, X and XI were solitary clusters, composed of only one accession in each cluster *i.e.* accession IC 356381 (from Uttar Pradesh), IC 118880 (Uttarakhand), IC567363 (Madhya Pradesh), EC 541202 (Russia) and EC 541216 (Russia), respectively. The result of the present study is similar to that of the previous reports. Verma (1999) evaluated 298 accessions and grouped them in 10 clusters. Khan *et al.* (2013) studied the genetic diversity of 55 genotypes and grouped them into 13 clusters. Sinha and Wagh (2013) grouped 33 linseed genotypes into three clusters.

The pattern of distribution of the accessions in different cluster further revealed that the clustering did not follow any particular pattern with respect to the origin of germplasm The accessions collected or originated from different parts of the world or different region within the country were grouped into same cluster and accessions of same region were grouped into different clusters (solitary clusters V, VI, X, XI and XII are exceptional cases). The result is an indication that the accessions belonging to same geographic origin might have passed different environmental conditions, adaptation, selection pressure etc.

4. Intra and inter-cluster distances: The inter- and intra-cluster distances (Table 3) reflect the interrelationship among clusters. The intra-cluster distance was in the range of 5.39 in cluster I to12.58 in cluster IX (excluding solitary clusters). Clusters V, VI, VIII, X and XI were represented by single accession each; hence their intra cluster distance was zero. The inter-cluster distance which has practical implication in breeding programme, was the farthest between cluster IX and cluster XII (D^2=67.61) and cluster VIII and XII (D^2=62.94). It is an indication of greater genetic divergence between Cluster IX and XII and between Cluster VIII and XII. Cluster IX contains six accessions namely, EC 541198, EC 541217, IC 398794, IC 397953, EC 541204, IC 267673. These accessions have their origin in Russia and India (Bihar, Himachal Pradesh and Jharkhand). Cluster XII contains two accessions namely, EC 541211 and EC 541214. Both were originated in Russia. Cluster VIII was composed of only one accession (IC 567363) collected from Madhya Pradesh. A crossing programme may be initiated between the accessions belonging to more divergent clusters *i.e.* between clusters IX and XII as well as clusters VIII and XII as it would provide greater opportunity for bringing together gene resulted probably due to complementary interaction of divergent genes in parents as suggested by Tadesse *et al.* (2010) and Khan et al. (2013).

An important point to be considered while selecting genotype for crossing programme from the genetically divergent clusters is that the *per se* performance of the accessions *i.e.* the particular accession selected should have high mean performance for the economically important traits. The chances of obtaining progenies with desired recombination will be more in such selection. In cluster IX, the accession EC 541198 (Russia origin) showed combination of relatively higher plant height (78.90 cm), higher seed yield (2.56 g/plant) and higher seed oil content (36.46%); IC 397953 (India origin-Himachal Pradesh) exhibited early flowering (days to 50% flowering =58 days) while the accession IC 267673(India origin-Jharkhand) possessed higher 1000 seed weight (4.20 g) and higher oil content (37.82%). Therefore, these three accessions (EC 541198, IC 397953, IC 267673) were the best choice out of 6 accessions in cluster IX. In cluster, the accession EC 541215 (Russia origin) and EC 541218 (Russia origin) showed equal performance for seed yield and oil content but the later accessions was taller (81.91 cm) than the former (75.64 cm). Hence, both the accessions (EC 541215 and EC 541218) are of good choice for hybridization programme.

The lowest D^2 distance was observed between cluster V and VI (D^2=3.98) and cluster X and XI (D^2=5.65) indicated close relationship or less genetic diversity between the clusters. Cluster V is composed of one accession namely IC 356381 and cluster VI is composed of one accession namely, IC 118880; Cluster X is composed of accession EC 541202 while cluster XI is composed of accession EC 541216. Therefore, crosses between the accessions may be avoided.

5. Cluster means: Mean performance of a cluster (Table 4) is the overall mean values of individual correlated variables of all the genotypes included in the cluster. The cluster means reflects the uniqueness of the genotypes in that cluster. In addition, breeders may choose parental lines from other clusters as well on the basis of cluster mean. Cluster X was showing high mean for seed yield (3.59 g/plant). Cluster II is showing high mean performance for oil content (37.95%). Cluster V is showing earliness (attains 50% flowering in 53 days). If one desire to breed fibre type flax then higher stalk length gain importance. The mean height of cluster XII was the highest (78.77 cm). It is possible to select high value accessions from the above clusters for direct exploitation as commercial varieties or for use as parents breeding programme.

Conclusion:

Knowledge on genetic diversity among germplasm accessions conserved in the gene bank is necessary to identify genetically divergent parents for hybridization programme, to establish core collections and to plan future germplasm exploration trips. The Mahalanobis D^2 statistics has been suggested by several workers in quantifying the degree of divergence between biological populations at genotypic level and also to assess the relative contribution of different components to the total divergence both at inter and intra cluster level. The same was employed in the present study. Hybridization between genetically divergent and high mean parents such as IC 567363 x EC 541215 and EC 541218 and EC 541198, IC 397953, IC 267673 x EC 541215 and EC 541218 is suggested to obtain useful segregants. In additions, the accessions IC 356381 (early flowering), EC 5412149 (taller stalk for flax fibre), EC 541212 (more capsules per plant), IC 118878 (high seed weight), EC 541202 (high seed yield) and IC 567363 (high oil content) may also be included in the crossing programme as their mean performance was satisfactory.

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Table 1. Contribution of different characters towards divergence.

Character	No. of times ranked 1st	Percent contribution	CV%
Oil content	148	2.82	18.43
1000 seed weight	196	3.73	2.40
Seed yield/ plant	398	7.58	32.84
Plant height	892	16.98	6.77
Days to 50% flowering	928	17.67	5.28
Days to maturity	1210	23.03	17.17
No. of capsules / plant	1481	28.19	1.68

Table 2. Clustering of 103 linseed accessions based on seven quantitative traits

Cluster No.	No. of accessions	Name of the constituent accessions	Geographical origin of the accessions
I	23	EC 001475, Garima, IC 118884, IC 118889, IC 118891, IC 118893, IC 208460, IC 212040, IC 283450, IC 320925,IC 332123, IC 346107, IC 356133,IC 356160,IC 356177, IC 356206, IC 356259, IC 356341, IC 374361, IC 397852, IC 424869, IC 424876, Kiran.	Australia, Bihar, Himachal Pradesh, Maharashtra, Punjab, Uttar Pradesh and West Bengal
II	19	EC 399082, EC 399084, EC 399085, IC 118877, IC 118888, IC 203213, IC 249015, IC 276955, IC 319845, IC 319846, IC 424547, IC 424873, IC 567321, IC 567352, IC 567357, IC 567364, IC 567430, IC 567432, IC 583756.	USA, Bihar, Himachal Pradesh, Jharkhand, Madhya Pradesh, Maharashtra, Uttar Pradesh, Uttarakhand
III	10	EC 541206, H-Local, IC 118879, IC 118882, IC 249013, IC 283385, IC 332112, IC 356297, IC 356385, IC 467931.	Russia, Bihar, Himachal Pradesh, Uttar Pradesh, Uttarakhand
IV	18	EC 541199, EC 541200, EC 541201, EC 541207, EC 541208, EC 541210, EC 541212, EC 541213, EC 541215, EC 541218, IC 054951, IC 118890, IC 212041, IC 249009, IC 249012, IC 268337, IC 356126, IC 356292.	Russia, Bihar, Madhya Pradesh, Maharashtra, Uttar Pradesh, West Bengal
V	1	IC 356381	Uttar Pradesh
VI	1	IC 118880	Uttarakhand
VII	20	IC 054947, IC 320984, IC 398796, IC 415665, IC 054949, EC 541203, IC 118878, IC 118886, IC 424875, IC 583757, IC 424874, IC 118850, EC 541205, IC 331580, IC 118883, IC 318868, EC 041762, IC 345393, IC 118887, IC 345391	Argentina, Russia, Bihar, Himachal Pradesh, Jharkhand, Maharashtra, Rajasthan, Uttar Pradesh and Uttarakhand
VIII	1	IC 567363	Madhya Pradesh
IX	6	EC 541198, EC 541217, IC 398794, IC 397953, EC 541204, IC 267673	Russia, Bihar, Himachal Pradesh, Jharkhand
X	1	EC 541202	Russia
XI	1	EC 541216	Russia
XII	2	EC 541211, EC 541214	Russia

Table 3. Average D^2 value depicting inter cluster distances (off-diagonal values) and intra cluster distances (diagonal values)

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9	Cluster 10	Cluster 11	Cluster 12
Cluster 1	5.39											
Cluster 2	9.91	6.79										
Cluster 3	12.38	15.00	8.27									
Cluster 4	14.34	21.68	19.89	10.30								
Cluster 5	11.03	16.48	27.59	14.51	0.00							
Cluster 6	10.76	10.40	27.73	22.02	3.98	0.00						
Cluster 7	15.11	15.93	30.54	25.23	10.21	7.69	12.31					
Cluster 8	15.20	10.15	27.52	36.26	19.41	7.48	18.17	0.00				
Cluster 9	12.82	21.06	17.18	24.19	24.41	25.20	32.30	23.17	12.58			
Cluster 10	18.33	16.93	15.70	15.82	26.68	29.03	27.47	38.84	34.13	0.00		
Cluster 11	19.60	14.77	11.16	21.04	32.51	31.16	32.31	34.70	32.60	5.65	0.00	
Cluster 12	47.22	36.79	39.00	35.48	52.37	53.04	54.28	62.94	67.61	13.55	14.39	6.01

Electronic Journal of Plant Breeding, 6(3): 848-854 (Sep 2015)
ISSN 0975-928X

Table 4. Cluster mean of seven quantitative traits in linseed germplasm

	Days to 50% flowering	No. of capsules / plant	Plant height	Days to maturity	1000 seed weight	Seed yield/ plant	Oil content
Cluster 1	61.34	68.91	53.14	110.52	4.82	2.61	36.82
Cluster 2	64.22	69.26	58.38	113.36	5.14	2.32	37.27
Cluster 3	67.64	64.34	56.41	112.75	4.27	2.62	35.83
Cluster 4	54.90	72.91	58.30	107.57	4.78	2.84	36.31
Cluster 5	53.00	70.90	52.08	109.00	6.18	2.68	37.14
Cluster 6	57.50	72.55	53.44	111.00	6.18	2.22	37.82
Cluster 7	57.90	68.21	54.05	109.74	6.20	2.88	37.84
Cluster 8	64.00	69.90	53.98	114.75	5.15	1.26	37.95
Cluster 9	62.71	70.41	49.71	112.42	3.80	2.17	36.16
Cluster 10	59.75	63.30	67.21	110.25	4.60	3.58	36.82
Cluster 11	68.50	70.70	65.31	113.75	5.03	3.10	36.06
Cluster 12	59.13	70.88	78.77	111.25	4.85	2.71	36.69
CV %	7.84	4.22	14.14	1.89	15.03	22.10	1.97