Assessment of genetic and geographic divergence in linseed (*Linum usitatissimum* L.) genotypes.

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Abstract

Mahalanobis D^2 statistic was used to study the genetic diversity between and within the seventy-three genotypes of linseed (Linum usitatissimum L.). Analysis of variance indicated highly significant differences among the genotypes for all the traits indicating the presence of adequate variability and the possibility to undertake cluster analysis. By adopting Tocher's technique, the 73 genotypes were grouped into 7 clusters, where cluster II was the largest containing 17 genotypes followed by cluster VII and V with 12 genotypes. The inter-cluster distance was maximum between cluster I and VI (25.593), which indicated that the genotypes included in these clusters could give a high heterotic response and thus better sergeants. The maximum cluster mean was revealed by cluster VI for days to 50% flowering, the number of primary branches per plant, and the number of secondary branches per plant. Cluster VII showed the highest value for days to maturity, the number of seeds per capsule, and 1000-seed weight.

Among the ten traits studied, days to 50% flowering contributed the most (28.99%) followed by seed yield per plant (20.85%) towards the divergence of genotypes.

Keywords — *cluster, D^2, genetic divergence, genotypes, linseed*

INTRODUCTION

Linseed (*Linum usitatissimum* L.) is one of the most important oilseed crops in terms of seed yield, oil yield, and fiber yield. Owing to its various uses, it plays a vital role in the oilseed economy of the country. The seed is primarily used for extracting oil, which is used in various industries. In technical oil production, linseed ranks first followed by castor in the country. Every part of the linseed plant is utilized commercially, either directly or after processing. About 80% of linseed oil production goes to industries which further utilized in paint, varnishes, printing ink, pad ink, soaps, patent leather, manufacture of linoleum oilcloth, and other products. The remaining 20% of linseed oil used as an edible oil in individual pockets of Madhya Pradesh, Bihar, Maharashtra, etc. Discoveries of the use of linseed oil in cementing of roads and in antibiotics have given it new importance. Recently it has emolument consideration of new interest for its emerging market value as a functional food for its high content in fatty acids, especially for alpha-linolenic acid (ALA) and lignin oligomers, which also constitute around 57% of total fatty acids in linseed (Ayhan, 2009; Gallardo *et al*., 2013). These fatty acids were found to lower the levels of tri-glycerids present in the bloodstream, thereby reducing heart diseases and also show promise in the battle against inflammatory diseases such as rheumatoid arthritis (Kristensen *et al.,* 2012; Reddy *et al*., 2013). The existing variability can be used to further enhance the seed yield of the cultivars by following appropriate breeding strategies. Before initiating any such venture, proper assessment of the existing variability and identification of genetically diverse genotypes for use in a breeding program is of immense importance as it influences the frequency of desirable recombinants and heterotic response. Among the various techniques proposed for choosing parents, the Mahalanobis D2 statistic is useful for qualifying the degree of divergence among genotypes. In the present study, an attempt was made to understand the nature and magnitude of genetic divergence and to select divergent parents for their hybridization programs.

I. MATERIALS AND METHODS

A. Plant material:

The study was conducted at Oilseeds Research Farm, Kalyanpur, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India (Latitude 26^0 28' N and Longitude 80^0 24' E) in the years 2016-2017. The experiment was laid out in a randomized complete block design with three replications. Seventy-three genetically diverse genotypes of linseed were planted on 20^{th} November,62016. Seventy-three genotypes having different geographic origins were selected from various parts of the linseed growing area of India. Each plot consists of eight rows of 5m long. The spacing between plants was 8 cm. Rows were 25 cm. Apart. Local commercial practices were followed for cultivation, fertilization, and disease control. Plots were harvested in net plot size 4.0 x 1.5 m^2.

B. Data Collection

Five randomly selected representative plants from each plot were used for various data collection. To gain an understanding of plant growth and development, various traits, namely days to 50% flowering and days to maturity, were recorded by the visual assessment on a single observation of a group of plants during the growing season. Other quantitative traits, namely plant height (cm.), 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 (g), and seed yield per plant (g) were recorded at harvest. Seed oil content was estimated using Nuclear Magnetic Resonance (NMR) technique as per the procedure described by Alam and Alam (2005). Seed oil content results were expressed as a percentage (%).

C. Statistical analysis:

Analysis of variance (ANOVA) for all the traits recorded was conducted using PROC ANOVA (Analysis of Variance Procedures) in SAS 9.4 statistical software package software (copyright 2015, SAS Institute, Inc., Cary, NC, USA). The genetic diversity was performed following Mahalanobis D^2 statistic (Mahalanobis, 1936), and the genotypes were grouped into different clusters according to Tocher's methods (Rao, 1952). The contribution of each trait for genetic divergence was estimated from the number of times each trait appeared in the first rank. The intra-cluster distance was calculated by taking the average of the component genotypes, as suggested by Singh and Chaudhary, 1977. The inter-cluster distance was arrived by considering all the component D^2 values possible among the members of the clusters. The values of the genetic distance between the clusters were arrived by taking the square root of the average D^2 values. All tables were depicted using Excel (Microsoft, Seattle, WA). A p-value <0.05 was considered significant.

RESULTS AND DISCUSSION

The seventy-three genotypes selected for the study and their geographic location of origin are shown in Table 1. The analysis of variance (ANOVA) showed significant differences between the genotypes for the 1000-seed weight (g), number of secondary branches per plant, plant height (cm.), days to 50% flowering, seed yield per plant (g), number of capsules per plant, days to maturity, number of primary branches per plant, number of seeds per capsule and oil content(%). The aggregate effects of all the ten traits were tested by Wilk's criterion, indicating a thereby wide range of genetic variability and scope for selection of these characters (Table 2). A significant variation for all these traits was reported previously (Tyagi *et al.*, 2014; Sharma *et al*., 2017; Paul *et al*., 2016). Hence, the analysis of genetic divergence based on D^2 values was considered relevant. The seventy-three genotypes were grouped into seven clusters. The cluster II was the most abundant comprising of 17 genotypes followed by cluster V and VII with 12 genotypes, cluster IV with ten genotypes, cluster VI with eight genotypes, and cluster I and III with seven genotypes (Table 3).

The clustering pattern indicated that there was no relationship between geographic distribution and genotypic diversity. The 45 genotypes from Kanpur, UP were spread over all the seven clusters likewise, five genotypes of Sagar (MP) origin fall in four clusters (cluster I, cluster IV, cluster V and cluster VII). Two genotypes of Ludhiana (Punjab) origin fall in cluster III and cluster VII separately. Further 12 genotypes were originating from different locations *viz;* Maurnipur (UP), Keonjhar (Odisha), Kota (Rajasthan), Raipur (CG), Kanpur (UP), Faizabad (UP), Nagpur (MS) and Sagar (MP) having different geographical conditions were grouped in one cluster V. These results showed that geographic diversity is not necessarily a direct cause of genetic diversity (Trehan *et al*.,1974). Genetic drift and selection in a different environment could cause greater variety among genotypes than their geographic distances. Frequent exchange of breeding materials from one place to another (Verma and Mehta, 1976) and other selection may also be responsible for the distribution of gene complexes over the distant geographical location. Thus, it is more appropriate to select genotypes for hybridization-based on genetic diversity rather than geographic diversity. These findings are similar to earlier workers (Mahto *et al*., 1995; Srivastava *et al.*, 2009 and Paul *et al*., 2016).

Genetic diversity is the prerequisite of genetic improvement for yield and yield contributing traits. Before initiating the crossing program, the selection of diverse parents must be given due weightage to generate better segregants. The average intra and inter-cluster distance (Table 4) revealed that the Intra-cluster D^2 value varied from 3.988 to 6.802. The maximum intra-cluster distance was recorded in cluster VI (6.802), which comprised eight genotypes followed by cluster III (6.340) with seven genotypes, and the least intra-cluster distance was recorded in cluster VII (3.988), represented by twelve genotypes. It is advisable to select parents for hybridization within-cluster VI having eight genotypes that showed the highest D^2 value.

Maximum inter-cluster distance D^2 values were observed between cluster I and cluster VI (25.593). Since these clusters have more inter-cluster distance among them, crossing between

these clusters is expected to realize higher heterosis. Similar attempts to get maximum diversity among different linseed genotypes have been previously reported by other researchers (Tadesse *et al*., 2009; Pali and Mehta 2015).

The diversity was also supported by the appreciable amount of variation among the cluster mean for all the traits (Table 5). Cluster VI recorded the highest mean value for days to 50% flowering, the number of primary branches, and the number of secondary branches per plant. Cluster VII showed the highest value for days to maturity, the number of seeds per capsule, and 1000- seed weight. Cluster III showed the highest value for seed yield per plant and the second-highest value for the number of primary branches per plant (7.33) and the number of secondary branches per plant (33.10)). Thus, it is concluded that genotypes in different clusters can be put into the central gene

pool and can be utilized for isolating desirable transgressive segregants for realizing higher response.

The contribution of individual trait towards the divergence (Table 6) indicated that days to 50% flowering (28.99%) contributed the maximum followed by seed yield per plant (20.85%), number of capsules per plant (14.34%), plant height (10.35%), 1000-seed weight (10.35%) and number of secondary branches per plant (7.68%). The other characters viz. number of primary branches per plant, number of seeds per capsule, days to maturity, and oil content recorded negligible contribution. These traits *viz*, days to 50% flowering, seed yield per plant, and the number of capsules per plant should be given more emphasis for further selection and choice of right parents for hybridization. Similar results were reported by Tewari *et al*. (2013) and Nagaraja *et a*l. (2010). Hence, it is observed that diversity could be utilized for improvement in linseed by crossing the best performing genotypes of different clusters, followed by selection in segregating generations.

CONCLUSIONS

In the present study, the maximum inter-cluster distance was found between cluster I and cluster VI (25.593), followed by clusters I and III (23.697). Crosses among genotypes of these clusters could have resulted in better hybrid vigor and transgressive segregation. Since quantitative traits *viz*, days to 50% flowering, seed yield per plant, and the number of capsules per plant contributed maximum towards the divergence, natural selection of these traits helps crop improvement.

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TABLE I
73 Genotypes and their geographic origin

S. No.	Collection Centre (City, State)	Geographic status	Genotypes	
			No.	Name
1.	Ranchi, Jharkhand	23°21'N 55°20'E	03	BAU777-12, BAU2K-2012, BAU11-8
2.	Sagar, M.P.	33°83'N 78°74'E	05	SLS 91, SLS 92, PKDL 141, PKDL 143, PKDL 144
3.	Kota, Rajasthan	$25^0$10'N $73^0$49'E	04	RL 10129, RL 10175,RL 28007, Meera
4.	Raipur, CG.	$21^0$25'N$81^0$63'E	03	RLC 140, RLC 141, RLC 241
5.	Ludhiana, Punjab	$23^0$50'N $75^0$54'E	02	LCP 11-102, LCP 11-13
6.	Keonjhar, Odisha	$21^0$1'N $85^0$11'E	01	OL 98-6-4
7.	Nagpur, MH.	$21^0$7'N $79^0$7'E	02	NL 255, NL 273
8.	Palampur, HP.	$31^0$6'N $77^0$10'E	02	KL 247, KL 271
9.	Mauranipur, U.P.	$25^0$14'N 79^0 11'E	03	LMS 2011-21, LMS 2011-1-26, LMS 2011-91
10.	Faizabad, UP.	$26^0$47'N $82^0$12'E	03	NDL 2011-01, NDL 2011-08, NDL 2011-26
11.	Kanpur, UP.	$26^0$28'N $80^0$24'E	45	PCL 43, PCL 55, PCL 57, PCL60, LCK 1009, LCK 1207, LCK 1213, LCK1215,LCK 1301, LCK 1302, LCK 1303, LCK 1304, LCK 1305, LCK 1306, LCK 1307, LCK1308,LCK 1309,LCK 1310, LCK 1311, LCK 1312,LCK 1313, LCK 1314, LCK 1315, LCK 1316, LCK 1317, LCK 1318, LCK 1319, LCK 1320, LCK 1321, LCK 1322, LCK 1323, LCK 1324, LCK 1325, LCK 1326, LCK 1327, LCK 1328, LCK 1329, LCK 1330,Sheela, Shikha, Rashmi, Shekhar, T-397, Parvati, Neelum

TABLE II
Analysis of variance (ANOVA) for different traits in linseed

Traits	MS(Rep)	MS(Treat)	MS(Error)
Days to 50% flowering	2.458**	2.290**	1.413
Plant height(cm)	1.792**	2.391**	1.138
Days to maturity	1.547**	2.150**	1.144
No. of primary branches/ plant	1.721**	1.761**	1.774
No. of secondary branches/plant	1.711**	2.708**	1.739
No. of capsules/plant	1.740**	2.180**	1.669
No. of seeds/capsules	1.354**	1.246**	1.649
1000-seed weight(g)	1.170**	2.824**	1.369
Oil content (%)	1.625*	1.112**	1.246
Seed yield/ plant(g)	1.111*	2.250**	1.348

* Significant at 5% confidence level.
** = Significant at 1% confidence level.

TABLE III
Distribution of seventy-three genotypes of linseed in different clusters

Clusters	No. of genotypes	Name of genotypes
I	07	PCL-57, BAU 777-12, SLS-91, PKDL 141, RL 10129, LCK 1303 and LCK 1317
II	17	BAU 2K-2012, LCK 1302, LCK 1304, LCK 1307,LCK 1308, LCK 1310,LCK 1311, LCK 1312, LCK 1313, LCK 1314,LCK 1320,LCK 1325, Neelum, LCK 1326, LCK 1328,LCK 1329 and Sheela
III	07	PCL 43,LCK 1213, LMS 2011-21, RL 28007, LMS 2011-1-26, Meera and LCP 11-13 ,
IV	10	PKDL 144, BAU 11-8, Shikha, LCK 1009,LCK 1323, LCK 1324, LCK 1301 LCK 1315, LCK 1316 and LCK 1327
V	12	LMS 2011-91, OL 98-6-4, RLC 140, LCK 1215, NDL 2011-08, NL 255, PCL 55, RLC 241, PKDL 143, LCK 1321, T-397 and NL 273
VI	08	KL 247, RLC 141, NDL 201-01, PCL 60, KL 271, RL 10175, Rashmi and LCK 1305
VII	12	Shekhar, LCK 1322, SLS 92, LCP 11-102, NDL 2011-26, LCK 1207, Parvati, LCK 1308, LCK1309, LCK1318, LCK1319 and LCK 1330

TABLE IV
Intra and Inter cluster (D and D^2) values of 73 genotypes of linseed

S.No.	I	II	III	IV	V	VI	VII
I	2.195 (4.818)	2.929 (8.579)	4.868 (23.697)	4.032 (16.257)	2.691 (7.241)	5.059 (25.593)	4.069 (16.557)
II		2.028 (4.113)	4.097 (16.785)	2.900 (8.410)	2.422 (5.866)	3.964 (15.713)	2.068 (4.277)
III			2.518 (6.340)	3.287 (10.804)	3.800 (14.440)	3.422 (11.710)	3.455 (11.937)
IV				2.301 (5.295)	2.934 (8.608)	2.943 (8.661)	2.897 (8.393)
V					2.474 (6.121)	3.944 (15.555)	3.258 (10.615)
VI						2.608 (6.802)	3.672 (13.484)
VII							1.997 (3.988)

Figures in parentheses represent D^2 values.

TABLE V
Cluster means of 10 traits

S.No	Cluster	Days to 50% flowering	Plant height	Days to maturity	Primary branches /plant	Secondary branches /plant	Capsules/ plant	No.of seeds /plant	1000-seed weight	Oil content	Seed yield/ plant
1	I	55.57	49.48	126.14	6.76	24.76	136.52	7.29	8.73	41.97	5.74
2	II	70.63	57.96	134.71	5.57	20.16	101.38	8.06	8.82	40.72	4.89
3	III	71.29	72.57	139.86	7.33	33.10	113.43	8.52	7.23	39.83	13.60
4	IV	77.57	80.10	136.20	6.63	28.13	128.90	7.83	7.02	40.90	5.42
5	V	61.75	56.75	137.36	6.50	21.28	120.78	7.81	5.60	40.55	6.16
6	VI	82.12	67.25	141.62	9.33	36.08	103.25	7.71	7.02	38.54	5.81
7	VII	71.19	63.39	145.70	5.83	25.50	111.19	8.97	9.20	40.76	5.88

TABLE VI
Percent contribution towards total genetic divergence

S. No.	Traits	Contribution (%)
1.	Days to flowering	28.99
2.	Plant height(cm)	10.35
3.	Days to maturity	0.79
4.	No. of primary branches/plant	3.84
5.	No. of secondary branches/plant	7.68
6.	No. of capsules/plant	14.34
7.	No. of seeds/plant	2.16
8.	1000-seed/weight(g)	10.35
9.	Oil content(%)	0.60
10.	Seed yield/plant(g)	20.85