

ESTIMATION OF PHENOTYPIC DIVERGENCE IN LINSEED (*LINUM USITATISSIMUM* L.) FOR YIELD-RELATED TRAITS UNDER CHANGED CLIMATE IN MID-HILLS OF NORTH-WEST HIMALAYAS

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Key words: Linum usitatissimum, Genetic diversity, Mahalanobis D²-statistics

Abstract

Thirty two genotypes were analysed for nine morphological traits in RCBD replicated two times in two consecutive years (E-I, E-II) to investigate the genetic diversity pattern. Field data of two consecutive years were initially subjected to analysis of variance. There were highly significant ($p \leq 0.01$) differences among the genotypes for all the traits at both environments except for seeds per capsule, indicating the presence of adequate variability among the genotypes and the possibility to undertake cluster analysis. Moreover, genotypes responded differently to change in the environmental conditions at the two environments, as genotype \times environment interaction mean squares were highly significant ($p \leq 0.01$) for all the traits. The phenotypic divergence and relative importance were estimated by multivariate analysis. The cluster analysis based on Tocher's method classified the genotypes into eight (E-I) and six (E-II) major groups of different sizes during both the years. The maximum distance was found between clusters V and VII (E-I) and between clusters IV and V (E-II). The genotypes from these clusters can be utilized for the improvement of linseed yield and obtaining good segregants in linseed breeding programs. In both the years, 1000-seed weight contribute maximum in E-I (93.55%) and E-II (91.33%) total genetic divergence between genotypes.

Introduction

Flax (*Linum usitatissimum* L., $2n = 2x = 30$), is an annual self-pollinated crop which is commercially grown as a source of stem fibre and seed oil. The species is believed to have originated in either the Middle East or Indian regions (Vavilov 1951) and spread throughout Asia and Europe, prior to its introduction into the New World (Soto-Cerda *et al.* 2013). Fibre flax cultivars are taller and less branched and are grown in the cool-temperate regions of China, the Russian Federation and Western Europe (Soto-Cerda *et al.* 2013). Oilseed type flax plants (linseed) are more branched and shorter than the fibre type and are grown over a wider area in continental climate regions of Canada, India, China, the United States and Argentina (Soto-Cerda *et al.* 2013). Flax seed oil is utilized for the fabrication of various biodegradable products such as high quality drying oil, paints, varnishes and linoleum flooring. In addition, interest in flax oil and seeds as food products has increased due to their health benefits. Flax seeds contain relatively large amounts of α -linolenic acid, an omega-3 fatty acid, which is considered essential for human health. Despite huge benefits of linseed, it is grown only in 2.5 M ha of area in the world with annual production and productivity of 2.1 M tonnes and 827 kg/ha, respectively. India ranks second in terms of area (0.3 M ha) after Canada, but third in terms of production (0.2 M tonnes) after Canada and China, contributing about 14.89 per cent to world acreage and about 6.56 per cent to world production (Anon. 2013-14). In Himachal Pradesh total area under linseed is 0.0012 M ha with a total production of 0.00032 M tonnes with 270.0 kg/ha average productivity (Anon. 2013-14).

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Linseed being an important oilseed crop has a very low average productivity in India as well as in Himachal Pradesh because of many reasons such as, narrow genetic base of the available varieties, cultivation in marginal lands and due to biotic and abiotic stresses. Low yield of this crop is attributed to non availability of improved varieties to suit the diverse agro-climatic conditions. To overcome the poor yield levels, development of high yielding varieties becomes the top priority. Improvement in any crop depends on the availability of wide genetic diversity. Identification of promising genotypes is very useful during breeding from initial parent lines to the final variety release. Therefore, it is essential not only to conserve the genotypes but also to explore the gene-pool of linseed for breeding purposes of well adapted, better quality and high yielding varieties.

Cluster analysis had traditionally been used to distinguish the accessions from each other, their relationships and to get useful information on estimates of genetic diversity. In order to get transgressive segregation, genetic distance between parents is necessary (Joshi *et al.* 2004). Through higher genetic distance between parents, the higher heterosis in progeny can be observed (Ahmed *et al.* 2014). Initial diversity assessments in flax were carried out using morphological parameters (Bibi *et al.* 2013). Flax germplasm collections contain thousands of accessions of *L. usitatissimum* and related species, of which, subsets were assessed for the extent of genetic diversity for morphological characteristics (Sivaraj *et al.* 2012, Tyagi *et al.* 2014, Dikshita and Sivarajb 2015).

The present studies were thus planned to estimate genetic diversity in linseed germplasm using cluster analysis on the basis of morphological traits and to identify the best parent lines for using in future breeding programme.

Materials and Methods

A total of 32 genotypes of linseed were used in the present study (Table 1). All the 32 genotypes, including three check, *viz.*, Bhagsu, Binwa and Baner, of linseed were raised at the experimental farm of the Department of Crop Improvement, CSK HPKV Palampur (H.P.), India, during two consecutive years of *rabi* season, 2010-11 (E-I) and 2011-12 (E-II), for recording the morphological data. At two environments, experimental layout was a randomized complete block design with two replications. Plot size was 1.90 m² (3 rows, 2.5 m long and 25 cm row spacing). Data were recorded on nine different quantitative traits namely, days to 50 per cent flowering, days to maturity, plant height (cm), number of primary branches per plant, number of secondary branches per plant, capsules per plant, seeds per capsule, 1000-seed weight (g) and seed yield per plot (g). Five competitive plants were tagged randomly from each genotype in each replication for recording field observations for all the traits except for days to 50 per cent flowering, days to maturity and seed yield per plot, which was observed on plot basis during both years. The data recorded for each genotype at each environment were subjected to statistical analysis. ANOVA was conducted in each environment to test significant differences among genotypes (Panse and Sukhatme 1985).

Homogeneity of error variance tests were conducted to determine if data from individual environments (E) could be pooled to evaluate G × E interaction using a combined ANOVA. The Homogeneity of error variances was tested with F-test or the 'variance ratio' test as described by Gomez and Gomez (1984). For the combined analysis, variation was partitioned into relevant sources of variation to test for differences among genotypes and for the presence of G × E interaction. The phenotypic divergence among the accessions was estimated by the multivariate techniques, as follow: Tocher's cluster analysis as described by Rao (1952), using Mahalanobis D²-statistics (Mahalanobis 1936) to measure the genetic distance.

Table 1. List of linseed genotypes (seed flax) and their parentage used in the study.

S.No.	Genotype	Parentage	S.N.	Genotype	Parentage
1.	KL-240	RLC-29 × NL-93	17.	KL-257	LC-2323 × KLS-1
2.	KL-241	Giza-7 × KLS-1	18.	KL-258	Janaki × Surbhi
3.	KL-242	Gaurav × KLS-1	19.	KL-259	KL-187 × Flak-1
4.	KL-243	RL-904 × Exotic-2	20.	KL-260	KL-210 × KLS-1
5.	KL-244	(RLS-29 × Jeewan) × Jeewan	21.	KL-261	KL-233 × KLS-1
6.	KL-245	Jeewan × KLS-1	22.	KL-262	Himalini × Flak-1
7.	KL-246	Him Alsi-2 × RLC-29	23.	KL-263	KL-223 × KL-224
8.	KL-247	Neelam × Nagarkot	24.	KL-264	LC-2232 × KLS-1
9.	KL-249	Surbhi fawn seeded (selection)	25.	KL-265	KL-168 × KL-224
10.	KL-250	DC-3	26.	KL-266	LCK-9826 × KL-221
11.	KL-251	DC-5	27.	KL-267	KL-223 × Flak-1
12.	KL-252	DC-7	28.	KL-268	Early selection Him Alsi-2
13.	KL-253	Chambal × KL-221	29.	KL-269	Medium selection Him Alsi-2
14.	KL-254	KL-223 × KL-221	30.	Bhagsu	RL-50-3 × Surbhi
15.	KL-255	KL-210 × KL-224	31.	Binwa	Flak-1 × SPS 47 / 7-10-3
16.	KL-256	KL-210 × Flak-1	32.	Baner	EC-21741 × LC-216

Results and Discussion

Homogeneity of variance tests were conducted to determine if data from individual environments could be pooled to evaluate $G \times E$ interaction using a combined ANOVA. For the combined analysis, variation was partitioned into relevant sources of variation to test for differences among genotypes and for the presence of $G \times E$ interaction. Pooling is valid only when the variances are homogenous. The equality of two variances may be tested by using F test or the 'variance ratio' test. Homogeneity of variances tests indicated heterogeneous error variance for each trait in each of the two environments and does not allow for a combined, across environment analysis (Table 2). Highly significant genotypic differences were found for all the characters studied in both the environments except for the seeds per capsule. Significant variation for all the characters was also observed by earlier workers (Diederichsen and Fu 2008, Sivaraj *et al.* 2012, Bibi *et al.* 2013, Tyagi *et al.* 2014). The genotype \times environment interaction was highly significant ($p \leq 0.01$) for all traits except for seeds per capsule, suggesting the relative genotypic values for these traits changed significantly over the years. Therefore, no pooled analysis was possible for these traits. Moreover, genotypes responded differently to change in the environmental conditions at the two environments, as genotype \times environment interaction mean squares were highly significant ($p \leq 0.01$) for all the traits (Table 2). This indicates that experiments at several environments are required for evaluating the diversity in linseed germplasm.

The cluster formation (Tocher's analysis) distinguished the genotypes into eight (E-I) and six (E-II) diversity classes of different sizes during both the years (Tables 4, 5 and Figs 1, 2), different members within a cluster being assumed to be more closely related in terms of traits under consideration with each other than those members in different clusters. During first year, distribution of different groups revealed that there were six genotypes in clusters I, III and VI, four in cluster II, five in cluster IV, two in clusters V and VIII and only one in cluster VIII. On the other hand, in second year, cluster I was the largest with eleven genotypes followed by cluster II with ten genotypes and cluster III with genotypes.

Clusters IV, V and VI had one genotype each. Different clustering pattern were also reported on linseed by some earlier workers (Srivastava *et al.* 2009, Kant *et al.* 2011, Khan *et al.* 2013).

The pairwise generalized squared distances (D^2) among the eight clusters is presented in Table 3. The average intra-cluster distance ranged from 0.00 (cluster VIII) to 21.40 (cluster VI), while inter-cluster distance ranged from 19.32 (between clusters I and VIII) to 248.64 (between clusters V and VII). The maximum distance was found between clusters V and VII ($D^2 = 248.64$). The second most divergent clusters were II and V ($D^2 = 212.20$) followed by clusters VI and VII ($D^2 = 188.60$). Fulkar *et al.* (2007) reported maximum inter cluster distance between cluster II and X, Tadesse *et al.* (2009) reported between cluster I and IV, cluster I and III and minimum for cluster VIII and IX and IX and X. Maximum segregation and genetic recombination is expected from crosses that involve parents from the clusters characterized by maximum distances. It is assumed that genotypes in clusters with minimum distances are closely related among themselves.

Sinha and Wagh (2013) grouped linseed genotypes into 3 clusters and suggested that intercrossing of genotypes from different clusters may help in obtaining new lines with higher yield. Genotypes tended to group together in separate clusters on the basis of low, moderate or high mean values for different traits (Table 5). Cluster means revealed considerable differences among the clusters. Cluster I comprised genotypes with greater plant height and low seeds per capsule. Cluster II consisted mainly of early maturing genotypes with less number of primary branches per plant. Cluster III comprised mainly of late flowering genotypes with shortest plant height and low yield potential. Cluster V mainly comprised genotypes with late maturity, high 1000-seed weight, low number of secondary branches per plant and capsules per plant. Cluster VII mainly comprised genotypes with maximum seeds per capsule and small in seed size.

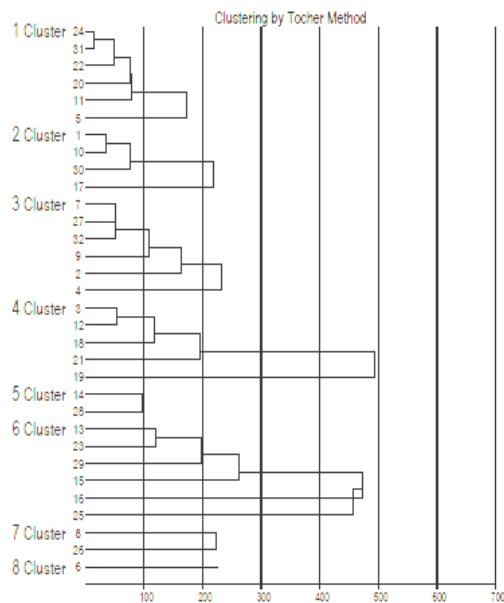


Fig. 1

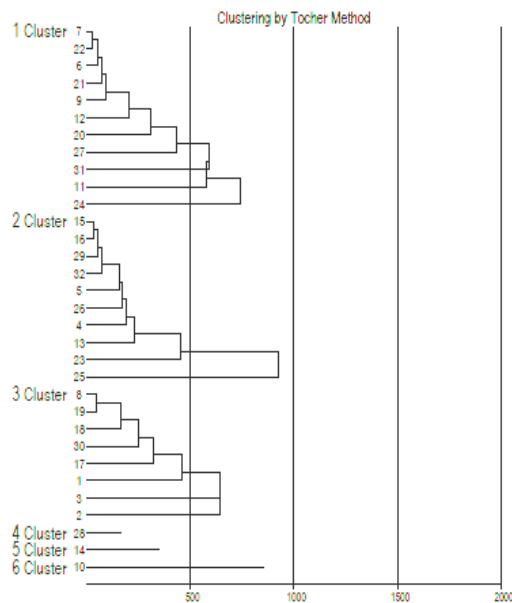


Fig. 2

Figs 1-2. Dendrograms showing grouping of 32 Linseed genotypes generated using D^2 cluster analysis (Tocher's method) in E-I and E-II.

Cluster VIII constituted superior genotypes for most of the traits *viz.*, high yield potential with early in flowering and high number of primary branches per plant, secondary branches per plant and capsules per plant.

Table 2. Univariate analysis of variance (ANOVA) for tests of significance of differences among genotypes, environments and their interaction, for nine traits in a collection of 32 linseed genotypes evaluated in two environments during 2010 - 2012.

Characters	E-I			E-II			Combined					
	Mean squares											
	Source	df	Genotypes	Error	Genotypes	Error	Genotypes	Environments	G × E	Pooled error		
Days to 50% flowering		31	46.29**	0.43	31	40.94**	1.61	31	59.11**	37709.45	28.13**	1.02
Days to maturity		31	6.32**	0.51	31	13.30**	0.13	31	11.18	2476.32	8.45**	0.32
Plant height (cm)		31	37.50**	15.96	31	75.38**	1.78	31	87.98**	2083.35	24.91**	8.87
Primary branches/plant		31	0.49*	0.26	31	0.39**	0.10	31	0.44	2.59	0.44**	0.19
Secondary branches/plant		31	3.21**	1.15	31	1.04**	0.20	31	2.9**	0.19	1.363**	0.68
Capsules/plant		31	64.29**	15.59	31	14.83**	1.09	31	47.29	689.13	31.83**	8.34
Seeds/capsule		31	0.64	0.58	31	0.12	0.10	31	0.29	0.95	0.46	0.34
1000-seed weight (g)		31	3.28**	0.001	31	1.90**	0.001	31	4.56**	17.08	0.63**	0.001
Seed yield/plot (g)		31	25307.86**	612.90	31	5509.68**	179.38	31	18952.32	200028.13	11865.23**	396.14

* and ** indicate significant at p value of 0.05 and 0.01 levels, respectively, G × E - genotype × environment.

Table 3. Intra- (bold) and intercluster divergence (D^2 values) among eight clusters of linseed during 2010 - 2011 (E-I).

Clusters	I	II	III	IV	V	VI	VII	VIII	Genotypes included in clusters
I (6)	11.29	114.58	31.67	63.94	98.35	40.74	150.91	19.32	KL-264, Binwa, KL-262, KL-260, KL-251, KL-244
II (4)	13.78	13.78	85.79	54.53	212.20	152.46	38.68	122.85	KL-240, KL-250, Bhagsu, KL-257
III (6)			13.78	37.55	127.58	68.99	122.16	42.67	KL-246, KL-267, Baner, KL-249, KL-241, KL-243
IV (5)				19.14	160.71	101.36	89.56	71.15	KL-242, KL-252, KL-258, KL-261, KL-259
V (2)					9.83	62.30	248.64	92.52	KL-254, KL-268
VI (6)						21.40	188.66	36.32	KL-253, KL-263, KL-269, KL-255, KL-256, KL-265
VII (2)							14.92	158.37	KL-247, KL-266
VIII (1)								0.00	KL-245

Table 4. Intra- (bold) and intercluster divergence (D^2 values) among eight clusters of linseed during 2011 - 2012 (E-II).

Clusters	I	II	III	IV	V	VI	Genotypes included in clusters
I (11)	22.24	44.49	50.63	76.87	87.50	94.67	KL-246, KL-262, KL-245, KL-261, KL-249, KL-252, KL-260, KL-267, Binwa, KL-251, KL-264
II (10)		20.50	87.32	39.76	49.01	133.31	KL-255, KL-256, KL-269, Baner, KL-244, KL-266, KL-243, KL-253, KL-263, KL-265
III (8)			23.60	121.86	132.49	50.44	KL-247, KL-259, KL-258, Bhagsu, KL-257, KL-240, KL-242, KL-241
IV (1)				0.00	18.67	168.29	KL-268
V (1)					0.00	179.44	KL-254
VI (1)						0.00	KL-250

Table 5. Cluster means for 32 genotypes studied for nine quantitative traits during 2010 -2011 (E-I).

Characters	I	II	III	IV	V	VI	VII	VIII	Mean	Minimum	Maximum
Days to 50% flowering	117.17	118.88	120.75**	112.90	117.00	114.92	114.50	105.50*	115.20	105.50	120.75
Days to maturity	206.42	204.75*	206.25	206.50	207.75**	206.42	206.25	206.00	206.29	204.75	207.75
Plant height	61.49**	59.43	57.23*	59.93	58.23	60.13	58.53	60.40	59.42	57.23	61.49
Primary branches/plant	3.87	3.50*	4.23	3.86	3.55	4.07	4.10	4.70**	3.99	3.50	4.70
Secondary branches/plant	4.83	5.18	6.08	5.13	3.50*	4.58	5.15	6.10**	5.07	3.50	6.10
Capsules/plant	19.58	21.63	24.58	23.40	16.25*	19.50	25.50	27.00**	22.18	16.25	27.00
Seeds/ capsule	7.00*	7.00*	7.25	7.00*	7.25	7.42	7.75**	7.50	7.27	7.00	7.75
1000-seed weight	7.51	5.27	6.95	6.25	9.44**	8.22	4.51*	7.56	6.96	4.51	9.44
Seed yield/plot	380.83	387.50	324.17*	439.00	330.00	338.33	402.50	580.00**	397.79	324.17	580.00

Table 6. Cluster means for 32 genotypes studied for nine quantitative traits during 2011 - 2012 (E-II).

Characters	I	II	III	IV	V	VI	Mean	Minimum	Maximum
Days to 50% flowering	132.50	132.17	131.25*	136.93	140.75**	136.75	135.06	131.25	140.75
Days to maturity	208.65*	210.63	211.17	211.71	212.25**	210.25	210.78	208.65	212.25
Plant height	60.62	63.64	63.18	65.16	74.05**	56.00*	63.78	56.00	74.05
Primary branches/plant	4.19	4.04	3.90*	4.15	3.95	4.60**	4.14	3.90	4.60
Secondary branches/plant	5.08	5.01	4.30*	5.46	4.70	7.60**	5.36	4.30	7.60
Capsules/plant	25.50	22.80	21.83*	25.36	24.50	33.25**	25.54	21.83	33.25
Seeds/capsule	7.29	7.35	7.35	7.12	7.00*	7.70**	7.30	7.00	7.70
1000-seed weight	5.26*	7.07	8.52**	5.83	6.85	6.53	6.68	5.26	8.52
Seed yield/plot	317.00	326.33	298.33	427.5**	225.00	192.50**	297.78	192.50	427.50

* Min. value, ** Max. value.

The diversity in the present materials was also supported by the appreciable amount of variation among cluster means for different traits (Table 6). Cluster I mainly characterised genotypes with early in maturity and small in seed size. Cluster III comprised genotypes with early flowering, low in number of primary branches per plant, secondary branches per plant, capsules per plant and bold in seed size. Cluster IV consisted of genotypes with high yield potential. Cluster V comprised genotypes with late flowering/ maturity and low seeds per capsule. Cluster VI consisted mainly genotypes with short plant height, maximum number of primary, secondary branches per plant, capsules per plant, seeds per capsule and low in yield potential. Factor responsible for the differentiation of all genotypes in different clusters during both the years at genotypic level attributed to the percentage contribution of quantitative traits towards genetic distance. In both the years, 1000-seed weight contribute maximum in E-I (93.55%) and E-II (91.33%) total genetic divergence between genotypes. Srivastava *et al.* (2009) in their study on genetic divergence reported that seed yield per plant contributed maximum towards genetic divergence followed by number of capsules per plant and days to flowering. The wide range of genetic diversity was observed in the present tested genotypes. It is also suggested that said diversity could be utilized for improvement in linseed by crossing best performing lines of different clusters, followed by selection in segregating generations.

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(Manuscript received on 13 October, 2015; revised on 9 November, 2015)