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Multivariate analysis in linseed (*Linum usitatissimum* L.)

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Abstract
The present study was conducted with 166 germplasm of linseed (*Linum usitatissimum*) including three checks, to assess the extent and pattern of genetic divergence. The experiment was conducted in Augmented Design II (AD-II) at Sam Higginbottom University of Agriculture, Technology and Sciences Allahabad (U.P) India during rabi 2016-2017. The genetic diversity was estimated by Mahalanobis D^2 statistics and the genotypes were grouped into 13 clusters using Tochers method each clusters having 15,3,11,20,4,11,14,17,16,19,15,9 and 12 genotypes respectively. The highest intra-cluster distance was found in cluster III followed by in descending order cluster II, IV, I and V, suggesting possibility of easy trait manipulation between these genotypes for linseed improvement. Cluster IV, XIII, VII and VI showing the highest mean for primary branches per plant number of capsule per plant, number of seeds per capsule and seed yield per plant, appears a desirable parent. Principle component analysis (PCA) showed the first four PCs had eigen value >1.00 and accounted more than 71.84% of total variation. The predominance nature of yield contributing traits *like* number of primary branches per plant, number of capsule per plant and number of seeds per capsule, indicated that these components were proven more important and crossing between and/ or within cluster may give good recombinants for linseed improvement.

Keywords: linseed, genetic diversity, principle component analysis

Introduction
Linseed (*Linum usitatissimum*) is an ancient crop that is widely cultivated as a source of fiber, oil and medicinally relevant compounds. Genetically linseed is self-pollinated diploid crop (2n=2x=30) and posses a relatively small genome. Yield components are the primary objectives under study for crop improvement as because Grafius (1978) [2] 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. Improvement in genetic architecture of any crop depends upon the nature and extent of genetic variability required to effect selection in any breeding material. Despite its importance, the area, production and productivity of linseed is still low, which may due to lack of high yielding varieties. It warrants an urgent development of some high yielding verities with determinate growth habit. Development of varieties with high yield and enhance the area of linseed without reducing the area of other crop(s). To develop such desirable genotypes, recombination breeding and trait manipulation offer the potential alternatives which however require suitable parents for crossing programme. Assessment of existing genetic diversity especially in the primary gene pool remains the most crucial factor in success any crop improvement programme. Among the various methods available to evaluate genetic diversity, multivariate analysis *like* D^2 statistics has been proven of enormous importance in various breeding applications particularly in selection of the most diverse genotypes appropriate for hybridization (Rahman and Al-Mansur, 2009) [3]. To understand variable independence and balanced weight of traits, principal component analysis (PCA) was performed, which resulted to an effective contribution of different traits on the basis of respective variation. Evaluation of germplasm is useful not only in selection of core collection but also its utilization in breeding programmes. The multivariate analysis, principal component and cluster analyses have been utilized for the evaluation of germplasm when studying various traits (Mehandi *et al*., 2015) [8]. In other words estimating the variability helps breeders to understand the evolutionary and genetic relationships among accessions and to select germplasm in a more systemic and effective way in their breeding program (Lavanya *et al*., 2008) [4]. Keeping the above points under consideration the present experiment was undertaken to assess the genetic divergence and isolate the suitable parents for hybridization and recombination breeding using multivariate cluster and principal component analysis.

Materials and Methods

One hundred sixty six germplasm lines of linseed including three checks were evaluated for various quantitative traits in augmented design II (AD-II) at Field Experimentation of Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences Allahabad (U.P) India during rabi 2014-2015. The experimental field was divided into five blocks. Each block consisted of thirty five germplasm lines of single row of 5 meter length spaced 30 cm apart with plant to plant distance of 10 cm. The checks were distributed randomly in each block. Recommended agronomic practices were followed to raise a good crop stand. All the quantitative traits were analyzed by numerical taxonomic techniques using the procedures of cluster and principal component analyses with the help of computer software Windostat Version 9.2. Cluster analysis was conducted on the basis of average distance Ward's method.

Results and Discussion

Study of genetic diversity in genetic resources is a critical factor for breeders to better understand the evolutionary and genetic relationship among accessions, to select germplasm in a more systemic and effective way and to develop the strategies to incorporate useful diversity in their breeding programmes.

Cluster analysis by tochers method

The mean sum of squares due to the genotypes were significant for all the characters studied except for days to maturity, suggesting the existence of high genetic variability among the genotypes for all the traits.

Selection of suitable parents plays an important role in any successful plant breeding program. Parents with more genetic distance are expected to exhibit higher genetic gains from selection. In the present experiment, the cluster analysis conducted in a set of one hundred sixty six genotypes generated a total of thirteen distinct clusters (Table 1). (Mehandi *et al*. 2015) [8] also gave emphasis on tochers method for effective selection of parental lines for crossing programme. Parents with more genetic distance will lead to higher genetic gains from selection

The largest cluster IV included twenty genotypes followed by Cluster X, VIII, IX, XI, I, VII, XIII, VI, III, XII, V and II and comprising nineteen, seventeen, sixteen, fifteen, fifteen, fourteen, twelve, eleven, eleven, nine, four and three, respectively suggesting existence of wide diversity among the genotypes used in the study. The highest intra-cluster distance was found in cluster III followed by in descending order cluster II, IV, I and V, suggesting possibility of easy trait manipulation between these genotypes for linseed improvement (Arunachalam, 1981) [1]. The results of the intra-cluster distance indicating that, the maximum amount of heterosis is expected in cross combination involving the genotypes of most divergent cluster.

The inter cluster distance varied from 12.20 to 27.75. It indicates that the genotypes in the cluster IX were more diverse than the genotypes in the above clusters. The maximum inter cluster distance was observed between cluster IX and XIII followed by cluster II and X. The minimum inter-cluster distance was observed between cluster V and X followed by cluster XI and XII. The cluster means of the quantitative traits helps to identify the diverse genotypes for genetic manipulation. Concerning days to maturity, all the clusters exhibited similar mean values thus suggesting common crop duration for the genotypes under study (Table 3). The genotypes of cluster XIII were responsible for highest cluster mean for days to 50% flowering followed by entries of cluster X. The genotypes with early flowering were concentrated in cluster III. The genotypes of cluster X were responsible for highest plant height and lowest for cluster XII, suggesting that these clusters could be considered for improvement of plant height of linseed. However, both positive and negative correlations of plant height with seed yield have been reported by various researchers. For example, Gul *et al*. (2008) [20] observed the negative association between plant height and seed yield per plant, whereas Rajan *et al*. (2001) [19], Lavanya and Toms (2009) [21] reported positive association between plant height and seed yield per plant. When, the plant height is used as selection criteria for yield improvement, the average intermodal distance and stem diameter must be taken under consideration but they were not included in the present study.

Since improvement in yield is the prime objective in any breeding scheme, cluster means for primary branches per plant, seed per capsule and its major components should be considered for selection of genotypes. Accordingly, cluster IV, XIII, VII and VI consisting of genotype IC-564608, L-80-1339, RL-11002 And GP(I)-27 showing the highest mean for primary branches per plant number of capsule per plant, number of seeds per capsule and seed yield per plant, appears a desirable parent. These observations are also in accordance with Ajit (2006) [9], for days to maturity (Mahto and Verma, 1998; Haque *et al*., 1994; Mahto and Singh 1996) [11, 13, 10], for plant height and number of capsules per plant (Mahto and Singh, 1996 and Mahto and Verma, 1998) [10, 11], for days to flowering and plant height (Asthana and Pandey, 1980), for capsules per plant and days to flowering (Verma, 1996) [15], for plant height (Chandra, 1977) [16], for number of capsules per plant (Mahto, 1999) [12]. The above results imply that in order to select genetically diverse genotypes for hybridization the material should be screened for important traits like days to maturity, plant height, number of capsule, number of seeds per capsule, days to 50% flowering, seed yield, test weight.

The results obtained with respect to per cent contribution of each character toward total diversity indicated that the characters like number of capsule/ plant, plant height and days to 50% flowering had maximum contribution towards the total divergence. Therefore, considering both cluster mean and per cent contribution of each character, genotypes belonging to cluster IV, XIII, VII and VI would be promising for use as breeding material in hybridization programme. Number of primary branches, number of seed/capsule, seed yield/ plant and test weight less contribution (<1%) which illustrates that the possibility of improvement of linseed targeting these traits is remarkably low (Table 4).

In order to generate transgressive segregates with higher yield, the genotypes in the above cluster may be targeted for multiple crossing programmes. In this way hybridization between genotypes of distant cluster will lead to accumulation of favorable genes in a single genotype. Furthermore it will also be helpful in generating new variability for developing the varieties involving a large number of different lines instead of closely instead of closely related ones. However, high diversity is required for getting higher number of recombinants but some of the reports are also available in support of crossing between moderately diverse genotypes to isolate the superior transgrassive sergeants. Shukla and Singh (2006) [17]; Yadav *et al.* (2007) [18] have also been observed the non-significant correlation between F_1 performance and their high parental diversity except some traits. Thus, the

moderately diverse genotypes can also be included in breeding programme to isolate the good recombinants. Therefore, at least one genotype from each cluster may be chosen and made crosses in diallel fashion may be efficient to isolate the good heterotic crosses and also helps to stabilize the relationship between genetic diversity and heterotic response.

Principal component analysis (PCA)

The progress in breeding programme for economic characters often depends on the availability of a large germplasm representing a diverse genetic variation. In order to ensure the efficient and effective use of crop germplasm, its characterization is imperative and multivariate analysis provides a good evaluation of landraces by identifying those that should be further evaluated at the genetic level (Rabbani *et al.*, 1998) [5].

The Eigen values are often used to fix the number of major principal components to be explained. The total of Eigen values is usually equal to the number of variables. Four principal components (PC1 to PC5) are extracted from the original data and having latent roots greater than one, accounting nearly 71.84% of the total variation as similar reported by Maqbool *et al*, 2010. The maximum eigen root value (1.53) was recorded for 1st PC, which explained 19.10% variation. The rest four PCs (2nd, 3rd, 4rd & 5th) explained 14.41%, 13.93%, 12.26% and 12.15% individual variation and 33.50%, 47.43%, 59.69% and 71.84% cumulative variation (Table 5).

The first PC was predominantly related to yield and yield contributing traits *like* number of primary branches per plant and number of capsule per plant, indicated that these components were proven more important towards the genetic diversity. Whereas the second PC contrasts variables that related solely to number of seeds per capsule with those that are also associated with yield. The third principal component was named of seed weight component since positively correlated with 1000 seed weight. The fourth and fifth principal component, accounted for 12.26% and 12.15% of the variation respectively. In this component, correlation of days to maturity and days to 50% flowering were highly positive. Because of that, this component entitled as factor crop duration. The results on PCA indicated that these traits are important for trait manipulation and diversity in this population was present due to these traits. Singh *et al.* (2014) [7] also gave emphasis on PCA for isolation of yield components with high diversity.

Table 1: Distribution of the 166 genotypes of linseed into different cluster

Cluster number	No. of genotypes	Cluster Members
I	15	EC-577M,EC-990020, NP-RR328, A-70(110), A-202B(184), 5620A(64), LCK-9312 (432), GP(I)-28, IC-564623, IC-564628, IC-564632, RL-10135, RL-10135-1, RL-10205, P(I)-243 and GPR-6
II	3	EC-104265, GPR-5, GPR-9.
III	11	EC-2082, EC-5328,NP-22, A-195(178),GS-40(990), GP(I)-38,GP(I)-215., GPR-2,GPR-12,GPR-13 and RLSPS-R-100
IV	20	1045(52),5/47-2/1/10/10(6),3-1-00000,KOTA-13(1266),GUHALOLAL(1141),GP(I)-23,GP(I)-24,GP(I) 47,BR-12(34),GP(I)-234,IC-564608,IC-564620,IC-564621,IDSW/RL-26015,RSTCR/20-RL-10193,RST®/8-RL-10106,RS(I)/RL-10108/9,RL-10166,RL-13191 and RL-13519
V	4	EC-577M,470Erg(47),RL-14509 and T-397 ©
VI	11	EC-577M,NP-RR10 (1634), GS-2346(1073), GP(I)-27, GP(I)-31, GP(I)-44, IC-564617, IC-564618,IC-564624,RL-14511 and Parvati ©
VII	14	A-24-1-2 (86), A-39(80), A-196(179), GP(I)-30, GP(I)-46, GP(I)-132, IC-564622, SUBRA, T-397, RST(I)/RL-10008-7. RL-11002,BB-34,GPR-11 and RLSPS-13505
VIII	17	EC-41528,A-12-1-12 (80), A-71(111), A-76(115), GP(I)-48,IC-564614,IC-564625,IC-564627,IC-564629,IC-564630, Pratapalsi -2,RST(I)A/RII/Meera,RL-13139,BB-64,GPR-4 and GPR-15,
IX	16	EC-41665,NP-RR191,A-4-1(74),A-73(113),A-181(175), A-203, 185), A-238(196), 12x15(17),GS-288(1091),GS-129,GP(I)-29, PADMINI, SHEKHAR, Neelam, RST(I)/RL-1012-7/18 and RLSPS-130519
X	19	EC-41656,IC-15866,A-60(100),A-370(209),417/1(3),11x17(16),JABALPUR 1986(12),IC-22794, GP(I)-27-1,1206(53),1937(59),4602(59,IC-564609,IC-564626,RL-130063,RL-10189,RL-29210,GP(I)-271 and RLSPS-130517
XI	15	EC-577M, EC-9900,NP-23K,A-40 (89),A-51 (95),A-72(112),A-202B(183),A-362(203,491(51),9*IBP-1986,GP(I)-71,GP(I)-101,IC-564612, RL-10103 and RL-13178
XII	9	EC-10077, NP-RR207,A-15-1-2 (81),A-459(265),GS-41(99) GP(I)-12,GS-139,17095.000,RL-10196 and RL-13502
XIII	12	EC-10077, EC-9900, NP-23K, A-40 (89), A-51 (95) A-72(112), A-199(181), A-202B(183), A-362(203, 491(51), 9*JBP-1986, L-80-1339, LCK-9310 (430), GP(I)-71, LCK-9313 (431), GP(I)-101, GP(I)-223, C-564611, IC-564612, IC-564631, RST(R)II/5-RL-101201, RL-10103, RL-13178, BB-109, RL-13006, RST(I) BRL-130543, SWETA

Table 2: Intra (Diagonal) and Inter Cluster Average Distances (D^2) for Different Quantitative Characters in Linseed

Cluster number	I	II	III	IV	V	VII	VII	VIII	IX	X	XI	XII	XIII
I	8.56	16.53	15.90	15.98	13.57	15.52	21.70	14.63	19.20	14.62	16.77	14.78	16.73
II		9.76	22.98	16.35	15.52	18.90	28.01	22.30	33.69	21.98	20.85	19.99	20.03
III			12.35	14.96	16.71	21.30	24.54	22.27	23.81	16.32	17.57	19.31	26.24
IV				8.96	12.20	19.48	15.83	16.40	19.49	14.19	15.51	13.38	19.28
V					6.97	14.65	14.81	18.92	24.25	12.65	14.30	17.24	17.22
VI						9.20	20.69	20.52	23.04	14.47	19.19	22.05	16.77
VII							11.04	15.12	18.66	13.29	16.64	19.44	23.90
VIII								8.01	16.03	12.62	14.56	13.36	21.16
IX									11.73	18.12	27.76	19.29	27.76
X										7.35	12.04	12.66	17.01
XI											7.14	17.09	18.36
XII												9.04	15.26
XIII													8.84

Table 3: Cluster mean values of thirteen clusters for different quantitative characters in linseed

	Days to 50% flowering	Days to maturity	plant height (cm)	No. of Primary Branches	No. of capsule/ plant	No. of seed/capsule	Seed yield/ Plant gm	Test weight
Cluster I	85.411	131.944	67.432	5.140	58.338	7.410	2.784	7.347
Cluster II	80.979	132.318	68.919	4.355	33.287	7.450	2.955	7.665
Cluster III	70.725	130.971	58.830	4.445	42.901	7.543	2.878	7.241
Cluster IV	78.333	132.667	66.093	3.700	15.200	7.673	2.538	5.068
Cluster V	82.667	129.000	85.293	3.867	21.967	7.540	3.851	7.518
Cluster VI	78.000	133.667	84.860	4.267	23.300	8.407	3.675	7.738
Cluster VII	78.450	133.667	75.248	5.612	76.228	7.782	3.154	7.226
Cluster VIII	72.667	134.000	88.893	3.867	22.967	6.540	3.841	5.498
Cluster IX	80.450	132.667	71.710	3.977	31.120	7.032	2.927	7.566
Cluster X	85.667	136.000	92.293	3.267	55.167	7.140	2.781	8.718
Cluster XI	68.667	136.000	48.893	4.467	76.767	6.940	4.091	7.788
Cluster XII	72.000	135.000	30.800	6.380	31.580	6.200	1.782	8.532
Cluster XIII	86.333	133.667	44.493	4.900	98.800	9.473	2.798	7.598

Table 4: Percent contribution of different quantitative characters towards genetic divergence

S. No.	Source	Contribution %
1	Days to 50% flowering	15.88
2	Days to maturity	1.57
3	plant height (cm)	23.30
4	No. of Primary Branches	0.13
5	No. of capsule/ plant	59.05
6	No. Of seed/capsule	0.04
7	Seed yield/ Plant gm	0.01
8	Test weight (gm)	0.03

Table 5: Eigen values, variability and Correlation coefficient of each agro-morphological trait with respect to its principle components (PCs)

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigene Value (Root)	1.53	1.15	1.11	0.98	0.97
% Var. Exp.	19.10	14.41	13.93	12.26	12.15
Cum. Var. Exp.	19.10	33.50	47.43	59.69	71.84
Days to 50% flowering	0.39	0.26	0.11	0.23	0.58
Days to maturity	0.30	0.13	0.26	0.71	-0.31
plant height (cm)	0.30	-0.34	0.42	-0.24	0.49
No. of Primary Branches	0.50	0.37	-0.04	-0.46	-0.11
No. of capsule/ plant	0.57	0.01	-0.20	-0.11	-0.41
No. Of seed/capsule	0.26	-0.57	-0.26	0.34	0.08
Seed yield/ Plant	0.08	-0.48	0.52	-0.22	-0.38
Test weight (gm)	-0.14	0.33	0.60	0.08	-0.09

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