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Assessment of Genetic Diversity using Mahalanobis D^2 Statistics in Lentil (*Lens culinaris* L. Medikus)

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ABSTRACT: Lentil is a valuable pulse crop with a high nutritional value and a high market price all over the world. Crop improvement largely depends on extend of genetic diversity in a crop species. In present investigation genetic divergence was assessed among 68 lentil genotypes consisting of released varieties and advanced breeding lines using Mahalanobis's D^2 analysis. Based on D^2 values genotypes were grouped into nine clusters. Most of the released varieties were placed in cluster I that possessed 15 genotypes, which implies that they share common parentage and have similar agromorphological features. Inter cluster distance was maximum between cluster VII and IX followed by cluster V and VII. It was found that the single genotype (PL406) present in cluster IX was highly diverse from other genotypes as inter cluster distance between cluster IX and most of the other cluster was relatively high.Thus, hybridization among these genotypes can generate desirable transgressive segregants. The characters *viz.* seed yield per plant, biological yield per plant and days to 50% flowering contributed maximum towards total divergence. Therefore, these traits should be considered in priority to characterize lentil germplasm.

Keywords: Genetic divergence, Lentil, D^2 analysis, cluster distance.

INTRODUCTION

Lentil (*Lens culinaris* L. Medikus) is one of the major grain legumes grown all over the world during cool season. Lentil is a diploid (2n=2X=14) self-pollinating crop with a haploid genome size of 4.3 Gbp (Kiristin *et al*., 2016). Globally, lentil ranks fourth in terms of production among the major pulses after dry bean, pea and chickpea. World lentil production was 6.33 million tonnes during 2019, that is approximately 8% of total dry pulse production. Canada is the world's leading lentil producer, followed by India. Together, Canada and India hold 65% share in world lentil production. In India lentil is grown during *rabi* season on an area of 1.56 million hectare with a total production of 1.56 million tones and a productivity level of 731 kg/ha (FAOSTAT, 2019). The current global lentil production is insufficient to meet demand, which is predicted to rise significantly as a result of the population growth and plant protein market. To close the demand-supply gap, efforts must be made to increase genetic gain, which is currently low due to narrow genetic basis of cultivated varieties. This is a significant impediment to creating cultivars for future demands (Rajendran *et al*., 2021).

Crop improvement depends heavily on the extent of genetic diversity in a crop species and the hybridization among the genetically diverse parents that produce transgressive segregants and greater variability in the successive segregating generations (Bohra *et al*., 2015; Pal *et al*., 2018, Meena *et al*., 2017; Singh, *et al*., 2017, and Verma *et al*., 2018). Thus, it is necessary to estimate genetic diversity by using morphological, biochemical or molecular markers. Plant breeders are interested in evaluating genetic diversity using morphological features since they are inexpensive, rapid and simple to score. The agro-morphological traits are an extremely useful tool for classifying and grouping of lines, as well as for studying taxonomic status and determination of genetic variation (Deep *et al*., 2019). Multivariate data analysis provides a graphic display of the multiple traits and genotypes in a way that can help in easy data interpretation. D^2 statistics is a quantitative measure of genetic divergence, where the clustering pattern of the genotypes is arbitrary. The Mahalanobis D^2 statistic helps in estimation of relative genetic divergence between genotypes and classify

them into homogenous groups or clusters. Tocher's method of grouping takes into account the full multidimensional space, even when the two canonical vectors justify high proportion of variation (Arunachalam, 1981). Considering the above points, the present study was undertaken to assess the genetic divergence among 68 genetically diverse genotypes of lentil using Mahalanobis D^2 Statistics.

MATERIALS AND METHODS

The present investigation comprising 68 genetically diverse genotypes of lentil was conducted in randomized complete block design (RCBD) with 3 replications during *Rabi* season of 2019-20 at Norman E. Borlaug, Crop Research Centre of the G. B. Pant University of Agriculture and Technology, Pantnagar (29.5° N and 79.3° E), India. The test plot consisted of 2 rows of 4-meter length with row to row and plant to plant spacing of 22.5 cm and 4-5 cm, respectively. The recommended package of practices was followed for raising a normal and healthy crop. The observations were recorded on five randomly selected competitive plants of each genotype from each replication on characters *viz.*, plant height (cm), number of primary branches per plant, number of secondary branches per plant, number of pods per plant, 100-seed weight (g), seed yield per plant (g), biological yield per plant (g) and Harvest index (%). However, the data on number of days to 50 per cent flowering and number of days to maturity were recorded on whole plot basis. The seed diameter (mm) was calculated as average length of 10 seeds placed in a row measured in centimeter scale. The mean values over plants were subjected for statistical analysis. Analysis of variance (ANOVA) for the observations recorded on different characteristics was carried out as per the standard procedure suggested by Panse and Sukhatme, (1995).Range for each character was worked out by depicting the lowest and highest values. The data collected on different characters was analyzed using 'Mahalanobis' D^2 analysis to determine the genetic divergence among the genotypes (Mahalanobis, 1936). The grouping of genotypes into different clusters was done using the Tocher's method as described by Rao (1948). Dendrogram was prepared using Indostat software.

RESULTS AND DISCUSSION

Knowledge of genetic diversity of germplasm is critical for active germplasm collection, conservation, documentation and utilization in crop improvement programme. In current investigation analysis of variance revealed highly significant differences among genotypes for all the characters studied indicating adequate genetic variability in the experimental material (Table 1). Presence of adequate amount of variability in material under investigation indicated the possibility of improvement in these characters by using suitable selection and hybridization program. The high amount of genetic variability for many of these traits has been earlier reported at morphological level (Ahamed *et al.* 2014, Chaudhary *et al*. 2017, Pandey *et al*. 2018 and Joshi *et al*., 2019); at biochemical level (Hammadi *et al*., 2021) and at genetic level (Sarvmeili *et al*., 2020; Chowdhury *et al*., 2020 and Dissanayake *et al.*, 2020).

Table 1: Treatment MMS, Mean and Range of traits under present investigation.

Sr. No.	Parameter	Mean	Range	Coefficient of variation (CV)	Treatment MSS
1.	Number of days to 50% flowering	80.22	73.00-90.00	1.63	32.78**
2.	Number of days to maturity	117.75	108.36-156.12	3.25	47.33**
3.	Plant height (cm)	29.59	19.31-48.21	10.51	42.94**
4.	Number of primary branches per plant	2.57	1.00-5.00	20.54	0.92**
5.	Number of secondary branches per plant	4.57	2.00-9.00	21.54	6.05**
6.	Number of pods per plant	41.34	16.34-90.54	22.35	648.47**
7.	Seed Diameter (mm)	3.84	3.01-5.24	6.93	0.55**
8.	100- seed weight (g)	2.05	0.75-3.40	15.94	0.75**
9.	Biological yield per plant (g)	1.78	0.36-3.90	14.30	1.48**
10.	Seed yield per plant (g)	0.65	0.12-1.94	16.75	0.43**
11.	Harvest index (%)	0.36	0.11-0.70	23.26	0.03**

**=significant at 1%, *=significant at 5%, MSS=Mean sum of squares

In the present study the genotypes were grouped into nine different clusters, indicating high genetic divergence among genotypes studied (Table 2). Apart from ecological and geographical diversity, this genetic divergence may be the result of a variety of processes such as shifting breeding material, genetic drift, natural variation, and artificial selection (Sirohi and Dar, 2009). Cluster I and Cluster II had the maximum number of genotypes *i.e.*, 15 each followed by cluster III (12); Cluster IV (10) and Cluster VIII (9). Cluster VI, VII and IX consisted only single genotype each (Fig. 1). It was observed that most of the cultivated released varieties viz. PL8, PL6, PL4, PL5, L4147, DPL15, PL 7 and LH 84-8 grouped together in the cluster I. These results indicated that the important released varieties in India share a narrow genetic base. Similar results were also reported earlier by Singh *et al*. (2005) and Khazaei *et al.*, (2016) in lentil.

Table 2: Clustering pattern among lentil genotypes.

Sr. No.	Number of genotypes	Genotypes
Cluster 1	15	PL 075, PL 056, IC 201738, PL 7, PL8, PL6, DPL15, IC201675, PL5, LL986, LH 84-8, L422, PL-165, PL4, L4147
Cluster 2	15	PL 017, PL 010, PL 029, PL 024, PL639, PL038, LL864, IC201798, PL057,LL1161, PL157, LL1207, ILWL118,ILWL248, IPL406
Cluster 3	12	PL 073, KLS 218, IC201648, IC207709, L4188, FLIP96-51, IC201707, PL083, IC279032, PL424, PL038, ILWL 118
Cluster 4	10	K 75, PL 15, IC396889, LL931, L4076, LL699, PL153, PL01, IPL321, LL 1114
Cluster 5	4	PL 17, LL875, PL234, IC 254371
Cluster 6	1	LH07-26
Cluster 7	1	DPL62
Cluster 8	9	DPL 58, PL046, PL030, LL931, LL1122, LL1208, L4710, PL107, LL1374
Cluster 9	1	PL406

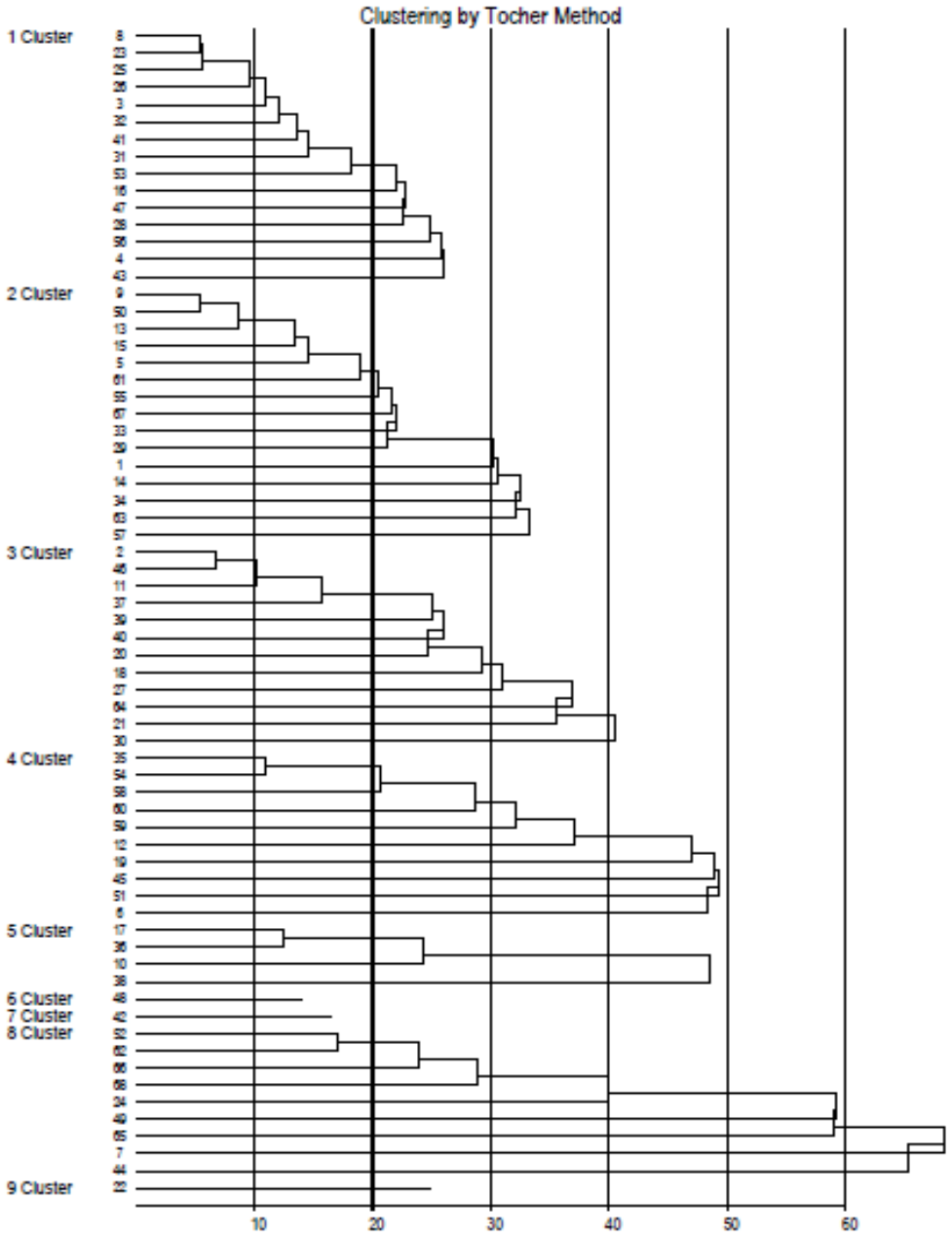

Fig. 1. Dendrogram showing the clustering pattern of different lentil genotypes.

A close perusal of Table 3 indicated that intra cluster distance ranged from 8.00 (cluster VIII) to zero (cluster VI, VII and IX). Maximum inter cluster distance was recorded between cluster VII and IX (18.32) which was followed by cluster V & VII (17.03) and cluster IV & IX (15.63). A critical perusal of Table 3 further revealed that the single genotype (PL406) present in cluster IX was highly diverse as inter cluster distance between cluster IX and most of the other cluster was relatively higher. As there is maximum genetic distance between the cluster VII and IX, the varieties grouped in these clusters viz. DPL 62 and PL 406 can be hybridized together to produce maximum genetic diversity in F_2 generation to obtain desirable genotypes and transgressive segregants in segregating generations. Arunachalum (1981) observed that the more diverse the parents are within their overall limits of fitness, the greater are the chances of heterotic expression of F_1s and a broad spectrum of variability in segregating generations. Joshi *et al.*, (2019) and Chaudhary *et al*., (2017) also advocated creation of genetic diversity to obtain desirable progenies in lentil. Inter cluster distance was found minimum between cluster I and Cluster IV which indicates that genotypes present in these clusters were genetically least diverse and almost of the same genetic architecture.

A close view of Table 4 indicated the contribution of each character towards the total divergence. It is clear from the table that seed yield per plant contributed maximum towards the total divergence (27.88%) which was followed by biological yield per plant (22.52%), days to 50 % flowering (22.21 %) and seed diameter (10.80 %). Therefore, these traits should be considered on priority bases for characterization of germplasm. Rest of the characters contributed very less and the contribution of harvest index towards the total divergence was least (0.79).

Table 3: Inter and intra – cluster distances in lentil.

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX
Cluster I	**4.76**								
Cluster II	6.71	**5.40**							
Cluster III	8.00	10.98	**5.92**						
Cluster IV	6.84	7.07	10.90	**7.02**					
Cluster V	9.91	13.05	7.25	12.03	**6.82**				
Cluster VI	5.89	6.66	7.41	8.26	10.24	**0.00**			
Cluster VII	9.37	6.54	14.20	9.52	17.03	9.05	**0.00**		
Cluster VIII	8.69	7.83	11.05	9.96	13.65	6.86	8.41	**8.00**	
Cluster IX	12.30	15.54	7.81	15.63	8.97	12.03	18.32	15.00	**0.00**

Table 4: Contribution (%) of different characters towards total divergence.

Sr. No.	Source	Times Ranked 1st	Percent contribution
1.	Number of days to 50% flowering	506	22.21
2.	Number of days to maturity	40	1.76
3.	Plant height (cm)	36	1.58
4.	Number of primary branches per plant	25	1.10
5.	Number of secondary branches per plant	112	4.92
6.	Number of pods per plant	44	1.93
7.	Seed Diameter (mm)	246	10.80
8.	100- seed weight (g)	103	4.52
9.	Biological yield per plant (g)	513	22.52
10.	Seed yield per plant (g)	635	27.88
11.	Harvest index (%)	18	0.79

Present finding regarding contribution of different characters towards total divergence are in perfect conformity with earlier findings of Chaudhary *et al.*, (2017) and Tyagi and Khan (2010). The diversity was also supported by the appreciable amount of variation among the cluster means for different characters (Table 5). Genotypes present in cluster I exhibited average value for all the studied traits. Cluster II possessed maximum mean value for seed diameter and had lowest value for harvesting index whereas, cluster III exhibited highest mean value for harvesting index. Cluster IV had lowest mean value for days to flowering and days to maturity suggesting that 10 genotypes present in this cluster can be used in breeding program to develop short duration early maturity varieties of lentil. Cluster V having four genotypes possessed high mean value for primary branches per plant, secondary branches per plant, pods per plant, 100 seed weight, biological yield and seed yield. Cluster VII and VIII possessed genotypes those were late in maturity and low yielding. Single genotype (PL406) present in cluster IX showed high mean yield for seed yield, biological yield and harvesting index. Such confirmatory results were also obtained by Roy *et al.* (2013) and Ahamed *et al.* (2014).

Table 5: Cluster means for different characters in lentil.

	DTF	DTM	PH	PBP	SBP	PPP	SD	100SW	BYP	SYP	HI
Cluster I	79.40	116.24	30.84	2.58	4.76	38.49	3.55	1.73	1.64	0.65	0.40
Cluster II	80.64	118.33	28.18	2.53	4.02	36.36	4.08	2.02	1.25	0.32	0.26
Cluster III	80.42	117.92	30.89	2.78	5.22	55.72	3.67	2.04	2.63	1.16	0.45
Cluster IV	76.73	118.77	29.00	2.37	4.23	33.67	3.89	2.24	1.27	0.38	0.35
Cluster V	76.33	112.00	31.92	2.75	5.83	59.83	4.02	2.56	2.92	1.20	0.42
Cluster VI	83.00	119.67	33.00	3.33	3.33	43.33	4.03	2.20	1.80	0.78	0.44
Cluster VII	84.67	122.67	27.33	2.00	4.67	29.00	3.94	1.54	0.47	0.14	0.36
Cluster VIII	85.15	119.93	27.56	2.56	4.00	31.85	4.06	2.32	1.75	0.54	0.30
Cluster IX	82.67	116.00	30.00	2.00	7.00	85.00	3.33	1.38	3.37	1.57	0.46

DTF= Days to 50% flowering, DTM= Days to maturity, PH= Plant height (cm), PBP= Number of primary branches per plant, SBP= Number of secondary branches per plant, PPP= Number of pods per plant, SD=Seed diameter (mm), 100SW= 100-seed weight (g), SYP= Seed yield per plant (g), BYP= Biological yield per plant (g) and HI= Harvest index (%).

Crosses made between highly divergent parents can be the most valuable for improvement in agronomic characteristics and higher productivity (Fu *et al*., 2014). Parents for hybridization can be selected based on the inter cluster distance and per se performance of different genotypes with respect to different traits. Based on the above finding's genotypes PL 8, PL 6, DPL15, PL4 and L4147 from cluster I; PL 7 and IC 254371 from cluster V and genotype PL406 from cluster IX were identified as donor for high yielding ability and yield contributing traits like number of pods per plant, primary branches per plant and secondary branches per plant. Genotypes LL875 and PL234 present in cluster V were identified as a potential donor for early maturity and high yielding ability. Genotypes PL030, LL1374, L4710 and DPL 58 from cluster VIII; genotype K75 from cluster IV and genotype PL010 from cluster II were identified as potential donor for increasing seed diameter. Therefore, progenies derived from hybridization including these genotypes are expected to show wide spectrum of genetic variability and a greater scope for isolating transgressive segregants in the advanced generations.

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