



Assessing genetic diversity among spontaneous mutant progenies of lentil (*Lens culinaris*) variety DPL 62

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ABSTRACT

An experiment was conducted during winter (*rabi*) season 2017–18, at the Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, to assess the genetic divergence for yield and yield component among 60 spontaneous mutant lines of lentil (*Lens culinaris* Medikus ssp. *culinaris*). Hierarchical cluster analysis grouped maximum numbers of genotypes in cluster II. Maximum intra cluster distance was exhibited by cluster II followed by cluster IV and cluster III whereas, maximum inter cluster distance was observed between cluster III and cluster VI followed by cluster V and cluster VI and cluster I and VI. Based on the high yield and yield contributing traits and their presence in distant clusters, the genotypes, viz. RKL 61F-2-15, RKL 58F-3715, RKL 1003-68G, Garima, RKL 1003-69G-A, RKL 23C-2741A and RKL 26C-345 can be effectively used in hybridization programme for lentil improvement to obtain desirable segregants. The first six principal component (PC) explain more than 65% of the total variability. Based on Principal factor (PF) scores, genotypes RKL 3-94, RKL 26C-340, RKL 50E-273, RKL 73GIII-13, RKL 51E, RKL 23C-2741 and RKL 26C-345 can be regarded as early maturing and high yielding. Genotypes, viz. RKL 61F-2-15, RKL 58F-3715, RKL 1003-68G, Garima, RKL 1003-69G-A, RKL 23C-2741A and RKL 26C-345 belongs to diverse clusters can be considered as better parents to be used in hybridization programme.

Keywords: Genetic diversity, Lentil, Mutants, Principal component, Variability, Yield

Lentil (*Lens culinaris* Medikus ssp. *culinaris*) is one of the principal pulse crops cultivated in semi-arid regions of the world, particularly in the Indian subcontinent and the dry areas of the Middle East. Nutritionally it is very rich and has the second highest ratio of protein per calorie, after soybean (Callaway 2004). India produced about 1.43 million tonnes of lentil from an area around 1.44 mha with average productivity of 10.09 t/ha during 2019–20 (Anonymous 2020). Although India ranks second in the world in respect of production (1.43mt) as well as acreage yet its position in average productivity of lentil is 23rd in the world which anticipates the scope of further improvement. One of the major concerns of low yield potential in lentil is narrow genetic base that restricts the further progress (Dikshit *et al.* 2015). Therefore, new variable sources need to be identified, developed and incorporated in the germplasm to make a breakthrough.

Lentil is a highly self-pollinated crop with negligible or no cross pollination, therefore, possibility of getting natural recombinant variables is very low. Spontaneous mutants, though occurring at a very low frequency, can be

a good option. The assessment of such genetic variation obtained through mutation and their behaviour with specific phenological responses provide the basis for adaptation to the climatic variables of the prevailing environment. A single plant with earliness and other morphological traits was isolated from a commercial population of DPL-62 in 2007–08, assuming it to be a mutant of DPL-62 by Punia *et al.* (2014). Progenies of this spontaneous mutant segregated up to sixth generation and wide variations were observed for morphological and seed traits. Segregation in further generations indicated a kind of dynamic mutation and by the sixth generations all the traits were almost fixed. Some of these mutant progenies were used in this study to ascertain information on genetic variability and divergence, assuming that it can play a crucial role in improvement of lentils.

MATERIALS AND METHODS

A total of 60 genotypes (56 progenies of a spontaneous mutant, DPL 62 with 4 checks) (Table 1) were grown in Randomized Block Design (RBD) with three replications during winter (*rabi*) 2017–18 at the research farm of CCS HAU, Hisar, Haryana. Each genotype was sown in a single row of 4 m length with 45 cm × 10 cm spacing. Observations were recorded on 22 quantitative and DUS traits. Morphological characters were recorded as per DUS Testing guideline on lentil (Singh *et al.* 2006).

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Table 1 Average inter and intra-cluster (diagonals) distance among the 60 lentil genotypes for yield and yield contributing traits

Cluster	I	II	III	IV	V	VI	VII	VIII
I	21.79							
II	64.91	44.21						
III	74.87	81.09	34.86					
IV	53.96	58.89	89.63	35.21				
V	101.53	85.76	54.25	104.48	30.42			
VI	143.60	107.17	168.36	106.49	167.21	-		
VII	109.63	71.78	69.55	107.43	59.43	128.42	19.62	
VIII	97.57	79.64	128.13	68.66	139.48	62.16	123.98	28.16

Statistical analysis: Hierarchical cluster and principal factor analyses of phenotypic observations were done using SPSS software (Version 2.0). UPGMA with City Block distance was used for clustering the genotypes (Romesburg 1990). Principal component method was used for factor extraction, and factor axes were rotated using Varimax rotation as all the variables could not be explained without rotation.

SSR based genetic diversity analysis: 50 SSR markers were selected for diversity analysis. Genomic DNA was isolated using CTAB extraction method (Saghai-Maroo *et al.* 1984). Quality and quantity of the isolated genomic DNA was estimated using agarose gel electrophoresis (0.8%). The gel was visualized in a UV transilluminator. Genomic DNA concentration varying from 50, 100 and 150 ng was checked in reaction volume of 10 µl. Best results were obtained at 50 ng DNA concentration.

RESULTS AND DISCUSSION

In the present study, 60 genotypes unveiled considerable amount of genetic variability for various traits except stem anthocyanin colouration and leaf pubescence which was in agreement with the previous studies by Gaad *et al.* (2017) and Hussan *et al.* (2018). The traits showing wide range of variation bestowed ample scope for adequate selection. The hierarchical cluster analysis grouped the genotypes into eight clusters (Supplementary Table 1). Maximum numbers of genotypes were grouped in cluster II followed by cluster IV, cluster III, cluster I and V, cluster VIII, cluster VII and cluster VI. The dendrogram represented the relative magnitude of resemblance among the different clusters.

Genotypes lying nearer to each other are more similar to one another than those lying apart (Fig 1). The discrimination of mutant lines into different discrete clusters indicated presence of substantial diversity in the material evaluated. Diversity studies in lentil genotypes were also reported by Mandal *et al.* (2014), Bhartiya *et al.* (2015) and Ahamed *et al.* (2016). Ahamed *et al.* (2016) grouped the germplasms of 110 lentil accessions into 10 clusters. The highest number of genotypes (17) was in cluster X and lowest (5) both in cluster II & IV.

The intra and inter-cluster analysis unveiled the information about the diversity. Maximum intra cluster distance was exhibited by cluster II followed by cluster IV and cluster III, while maximum inter cluster distance were observed between cluster III and VI followed by cluster V and cluster VI; cluster I and VI and cluster V and VIII (Table 1). The genotypes within the cluster exhibited less genetic diversity than different cluster with respect to aggregate effects of 20 characters studied. Cluster VI, represented by a single genotype, was most distant from other clusters, exhibited earliness for days to flowering and maturity, dwarf plant height, more number of branches/plant and pods/plant and large seeds but low seed yield. Cluster VIII, represented by 3 genotypes recorded with early maturity, highest seed yield/plant, more branches/plant and pods/plant (Supplementary Table 2). Cluster II having maximum members exhibited highest mean for seed yield/plant and 100-seed weight. Cluster V unveiled genotypes with long duration and broad seeds. Genotypes representing cluster I and IV had longest leaflet length, widest leaflet

Table 2 Total variance explained by different principal components

Principal component	Total Variance Explained					
	Without rotation			With Varimax Rotation		
	Eigen value	Per cent of variance	Cumulative %	Eigen value	Per cent of variance	Cumulative %
1	4.129	20.646	20.646	3.510	17.550	17.550
2	3.145	15.726	36.372	2.928	14.638	32.189
3	1.610	8.051	44.423	2.057	10.285	42.474
4	1.545	7.727	52.150	1.614	8.072	50.546
5	1.382	6.912	59.061	1.502	7.509	58.055
6	1.269	6.343	65.404	1.470	7.350	65.404

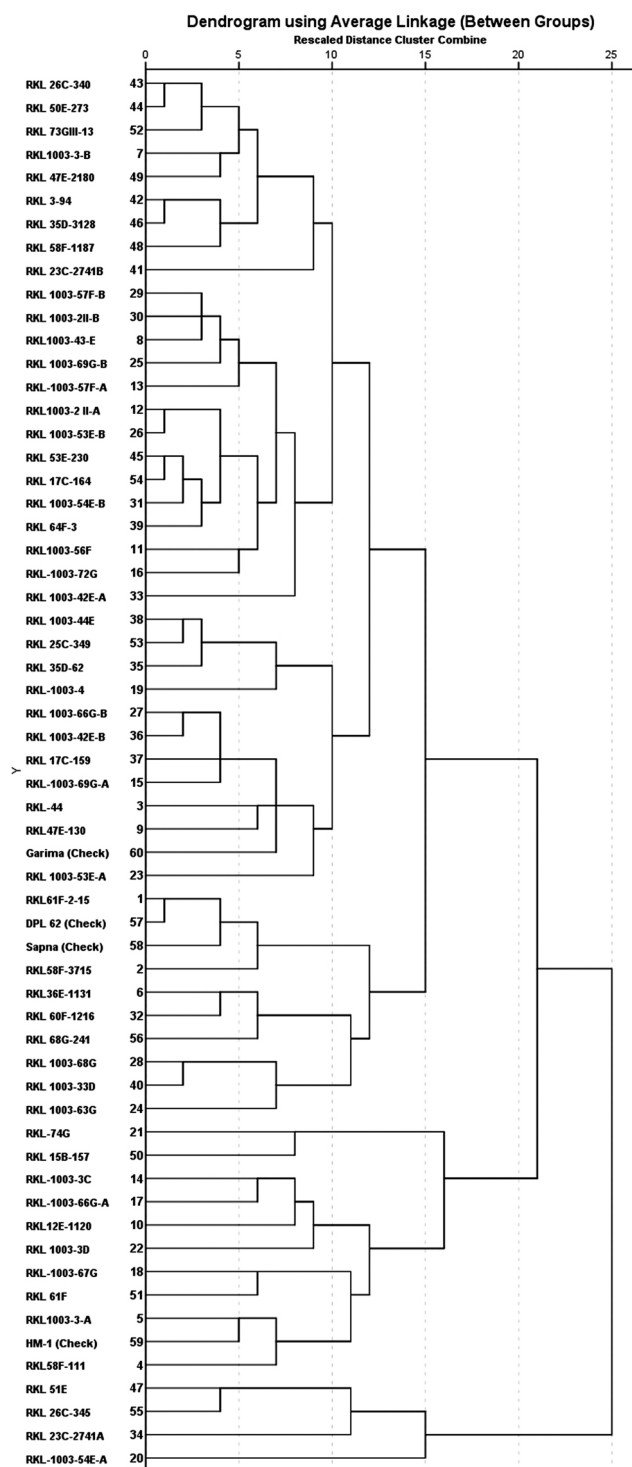


Fig 1 Dendrogram representing clustering pattern of 60 lentil genotypes.

breadth and also delayed flowering and maturity. Cluster VII having the highest mean for 100-seed weight but simultaneously exhibiting the lowest mean for seed yield/plant. Such clustering studies were also reported by Ahamed *et al.* (2016), Gaad *et al.* (2017), Gaur *et al.* (2020) and Khatun *et al.* (2022). Maximum mean value for peduncle length, plant height and number of pods/plant with the lowest mean value for days to flowering as well as days to maturity

was observed in Cluster V. Early maturing genotypes with higher yield were grouped into cluster VII. Based on the high yield and yield contributing traits and their presence in distant clusters the genotypes can be effectively used for further breeding program.

Factor analysis was carried out using principal component (PC) method (Table 2). The 1st PC explained 17.55% and 2nd, 3rd, 4th, 5th and 6th PCs explained 14.64, 10.86, 8.07, 7.51 and 7.35% variation, respectively. Bhartiya *et al.* (2015) carried out PCA for agro-morphological traits and reported that, first 4 PCs expressed 83.50% of total variation, whereas Katiyar and Kant (2015) got 7 PCs explaining most of the variation. Similarly, Nalia *et al.* (2019) evaluated lentil accessions using seed traits and revealed substantial contribution to the PC1. Further, Euclidean distances based on non-hierarchical cluster analysis were computed using these PC scores.

Factor loadings with Varimax rotations clearly indicated high loading for days to flowering, days to maturity, leaflet length, breadth and plant height on the PC1 while PC2 showed high loading for seed width, 100-seed weight, peduncle length, seed coat and cotyledon colour. The number of branches/plant, seed yield/plant, plant growth habit and number of pods/plant were highly loaded on PC3 while, seed coat pattern that on PC4. The PC5 was loaded by pod colour and foliage colour intensity while, PC6 with leaf tendril type, flower colour and seed profile. High loadings of different traits on a particular PC indicated strong association and these new factors could be used as selection criteria in lentil breeding programmes. Using PF score, genotypes were plotted for PC1 and PC3. These two PCs cumulatively accounted for seed yield and phenological characters (Fig 2). The plot clearly indicated the separation of early maturing dwarf genotypes towards the negative side of PC1 axis, whereas, genotypes with better yield characters separated towards the positive side of PC3 axis. Based on this, genotypes RKL 3-94, RKL 26C-340, RKL 50E-273, RKL 73GIII-13, RKL 51E, RKL 23C-2741 and RKL 26C-345 can be regarded as early maturing and high yielding. The genotypes found to be superior using factor analysis were also found to be members of the best performing one i.e. cluster VIII. Such confirmatory results were also obtained by Avtar *et al.* (2006) and Jindal *et al.* (2018) in cowpea.

SSR markers are considered as an important tool for studying genetic diversity thereby aiding in genetic improvement of crop plants (Hendre *et al.* 2007). Only 15 primers showed polymorphism out of 50 SSRs primers. Absence of amplification may be due to mutation at the primer binding site (Gupta P and Varshney R 2000). Two markers, viz. SSR 33 and SSR 48 showed polymorphism. Roy *et al.* (2015), Yadav *et al.* (2016), Bakir and Kahraman (2018) and Khatun *et al.* (2022) were also carried out diversity analysis in different lentil accession using SSRs and reported polymorphism. Roy *et al.* (2015), Khatun *et al.* (2022) and Gleridou *et al.* (2022) also reported maximum number of alleles per locus from SSR 33 which revealed polymorphism in our study.

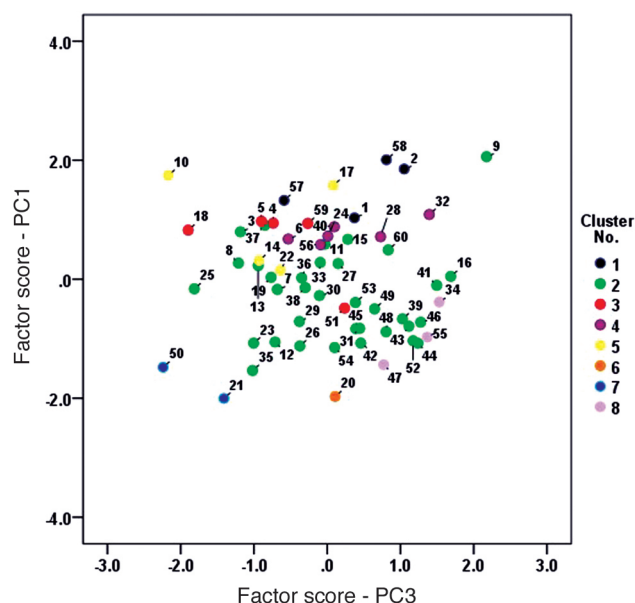


Fig 2 Location of 60 lentil genotypes based on PF scores wrt PC 1 and PC 3.

PF, Principal factor; PC, Principal component.

Remarkable genetic variability and diversity was observed among progenies of a spontaneous mutant of DPL 62 variety of lentil. Considering yield attributes the genotypes, viz. RKL 61F-2-15, RKL 58F-3715, RKL 1003-68G, Garima, RKL 1003-69G-A, RKL 23C-2741A and RKL 26C-345 were found superior and diverse. These can be considered as better parents for future hybridization programme to obtain desirable transgressive segregants.

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