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Genetic diversity analysis for quantitative traits in indigenous germplasm of lentil in Madhya Pradesh

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Abstract
Genetic divergence was assessed among 139 germplasm lines of lentil collected from different districts and tribal areas of Madhya Pradesh using Mahalanobis's D^2 analysis. The 139 lentil genotypes were grouped into 5 clusters. Cluster I was found to be the largest comprising 124 genotypes, followed by cluster III having 12 genotypes. Cluster II, IV and V had single genotype each. The pattern of distribution of genotypes from different eco- geographical regions into various clusters was at random indicating that geographical diversity and genetic diversity were not related. The characters *viz*. number of seeds per plant, days to maturity and total number of pods per plant contributed maximum towards genetic divergence among the genotypes and supposed to play important role in the improvement of lentil. On the basis of cluster mean, intra and inter cluster distance and per se, cluster III, IV and V may be used for their desirable characters in breeding programme of lentil. Entries like, JLC 54, JLC 117, JLC 132, JLC 123, JLC 113 and JLC 35 were selected which could be intercrossed to obtain high heterotic effect and also to recover desirable transgressive segregants.

Keywords: Masoor, Genetic divergence, D^2, Cluster analysis

Introduction
Lentil (*Lens culinaris* Medik) with 2n = 14, is India's fourth most important cool season, bushy annual pulse crop, locally known as "masoor". Lentil have the third highest level of protein from any plant-based food after soybean and hemp and is an important part of the diet in many parts of the world, especially in Indian subcontinent which have large vegetarian population. It is one of the principal crops cultivated in semi arid regions of the world, particularly in the Indian sub-continent, and the dry areas of Middle East. Globally, lentil shows only 5-6% of the total area under pulses. It is predominantly grown in Asia, which accounts for 80 – 95% global area and production (Malik, 2005) [1], respectively.
In India, lentil is being grown in 1.56 M ha area with production 1.06 M t and productivity 678 kg/ha. Uttar Pradesh, Madhya Pradesh, Jharkhand, Bihar and West Bengal are the major lentil growing states in India, sharing 85% and 90% of the total area and production, respectively. Madhya Pradesh covers 5.74 Lakh ha area with production 3.01 Lakh tones and productivity 531 kg/ha.
The major constraints behind the low productivity of lentil in Madhya Pradesh are lack of suitable varieties, poor crop establishment, diseases (rust, wilt and root rot), weed menace, terminal drought, poor microbial activity and non availability of quality seeds.
Improvement in a crop depends on the type and extent of variability in the desired character in the base material. Information on the nature and magnitude of genetic divergence in the population helps in choosing the diverse parents for meaningful hybridization (Gautam *et al*., 2013) [2]. The study of genetic diversity among genotypes is helpful in formulating effective crop breeding strategy. Genetic divergence has been studied in lentil (Sultana *et al*., 2005; Tyagi, and Khan, 2010; Asghar *et al*., 2010; Roy *et al*., 2013; Gautam *et al*., 2014) [3-7] and various other crops like sesame (Tripathi *et al*., 2013) [8], okra (Barche *et al*., 2014) [9] and mustard (Shekhawat *et al*., 2014; Kumar *et al*., 2013a & 2013b) [10, 12].
The importance of cluster analysis to determine the extent of variability has been reported before (Mahalanobis, 1936) [13]. D^2 statistics is a quantitative measure of genetic divergence, where the clustering pattern of the genotypes is arbitrary. The classification using generalized distance is functional, where the number of entries is not very large. (Vairavan *et al*., 1973) [14] used canonical analysis for initial grouping of a large number of rice germplasm collections. But simple two-dimensional representation of multidimensional disposition of genotypes cannot be as precise as the 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) [15].

Metroglyph analysis has been used in many crops as a method for initial grouping of the genotypes (Anderson, 1957) [16]. In the present study, therefore 139 germplasm lines were collected from different districts and tribal areas of Madhya Pradesh with the objective of conservation of variability for their further used in varietal development/ improvement.

Materials and Methods

The present study was carried out at the Seed Breeding Farm, Department of Plant Breeding and Genetics, JNKVV, Jabalpur. One hundred thirty nine germplasm lines collected from different districts and tribal areas of Madhya Pradesh were used as an experimental material. Observations were recorded on randomly selected five plants from each entry for ten economic traits *i.e.* days to maturity, plant height, number of primary branches per plant, number of secondary branches per plant, total number of pods per plant, number of effective pods per plant, number of seeds per plant, number of seeds per pod, 100-seed weight and seed yield per plant. The days to maturity were recorded on a whole plot basis and other traits are observed individually by selecting five random plants. Genetic diversity was estimated by calculating Mahalanobis (1936) [13] D^2 statistic. The genotypes were further grouped into different clusters as per Tocher's method (Rao, 1952) [17].

Results and Discussion

The analysis of variance revealed significant differences among the genotypes for all the characters studied indicating adequate genetic variability in the experimental material (Table 1). Genetic divergence among lentil germplasm lines was determined for seed yield and its attributing traits. The significant estimates of 'V' statistics during the analysis revealed significant differences among mean values of different correlated variables, thus analysis of genetic divergence among the tested lentil germplasm was considered to be relevant. Hierarchical cluster analysis based on agro morphological traits allocated the 139 lentil germplasm into five clusters (Fig.1). Critical assessment of clusters showed that clusters were heterogeneous within themselves and between each other based on major character relations. The composition of clusters and values of inter and intra clusters distances are given in tables 2 and 3, respectively. The results revealed that the inter cluster distance in most cases was larger than intra cluster distance suggesting wider diversity among the germplasm of different groups. Cluster I possessed the maximum number of 124 genotypes, followed by cluster III (12) in such a way that germplasm lines having minimum genetic distance were grouped in same cluster and *vice versa*. Rest of the clusters was mono-genotypic one.

The distribution of genotypes from different eco-geographical regions into these clusters was apparently random (Jeena and Singh, 2002) [18]. Genotypes of similar origin were grouped into different clusters and *vice versa*, thereby indicating non-relationship between geographical and genetic diversity. This tendency of genotypes to occur in clusters cutting across geographical boundaries demonstrates that geographical isolation is not the only factor causing genetic diversity (Sihag *et al*., 2004) [19]. This also suggests that the genotypes within cluster may have some degree of ancestral relationship. Similar findings were also reported by (Sirohi *et al*., 2007) [20] and [Kumar *et al*., 2004] [21]. The genetic divergence is an outcome of several factors such as changing of breeding material, genetic drift, natural variation and artificial selection other than ecological and geographical diversification (Sirohi *et al*., 2009) [22]. Therefore, selection of parents for hybridization should be based on genetic diversity rather than geographic diversity to get more heterotic recombinants and desired transgressive segregants. However, caution should be taken in selecting divergent genotypes because such crosses may not yield proportionate heterotic response (Subhash chandra *et al*., 2009) [23]. Therefore, a hybridization programme may be initiated involving the genotypes belonging to diverse clusters with high means for almost all component traits. Furthermore, these divergent parents should have better combining ability to give results proportionate to heterotic response.

The intra cluster average D^2 values ranged from 447.79 to 725.55 (Table 3). The highest intra cluster distance (725.55) was observed in the cluster I, indicating wide genetic variation among the genotypes included in the cluster. It is reported that genotypes would produce more desirable breeding materials for achieving maximum genetic distance with regard to yield *per se*, provided that there is adequate complementation of gene effects of parental lines (Rahman *et al*., 1997) [24]. Therefore, genotypes from cluster I should be given emphasis, while selection of parents for hybridization programme since most of the elite breeding cultivars were included in this cluster.

The inter cluster D^2 value ranged from 359.47 to 9438.10. Minimum inter cluster D^2 value was obtained between clusters III and II (D^2=359.47) indicate that the genotypes of these clusters were genetically least diverse and almost of the same genetic architecture. Such genotypes can also be used in breeding programmes for developing biparental crosses between the most diverse and closest groups to break the undesirable linkages between yield and its associated traits (Haddad *et al*., 2004) [25].

Maximum inter cluster D^2 value was observed between cluster V and I (D^2=9438.10), followed by cluster IV and I (D^2=8222.10), cluster V and II (D^2=4169.85) and cluster V and III (3345.61), revealing that genotypes included in these clusters are genetically diverse and may give rise to high heterotic response (Rama, 1992) [26]. Similar results were also found by (Qian and He, 1991) [27].

Cluster means of germplasm for ten characters in lentil (Table 4) revealed that cluster V had maximum number of primary branches per plant (3.17), number of secondary branches per plant (7.17), total number of pods per plant (163.0), number of effective pods per plant (139.0), number of seeds per plant (231.80) and seed yield per plant (4.53 g). Cluster IV reported to be highest number of seeds per pod (2.43), whereas cluster I early maturing average plant type with highest 100- seed weight (2.29g). These clusters can be preferred in selecting germplasm lines for respective traits as they recorded good means.

The characters contributing maximum divergence needs greater emphasis for deciding on the clusters for the purpose of selection of parents in the respective cluster for hybridization. The number of times, each of the yield component character appeared first in rank and its respective per cent of contribution towards genetic divergence was presented in table 5. The results showed that number of seeds per plant (47.01%) contributed highest towards genetic divergence by taking 4509 times first rank, followed by days to maturity (25.16%) by 2413 times, total number of pods per plant (13.44%) by 1289 times, 100-seed weight (5.79%) by 555 times and plant height (3.96%) by 380 times.

Principle component analysis was also conducted and presented in table 6. It showed association in PC1 with

number of seeds per plant, PC2 with plant height and days to maturity, PC3 with total number of pods per plant. Thus, re-structuring plant type with early flowering, more number of number of seeds per plant and total number of pods per plant would obviously generate plants with high seed yield in lentil.

Table 1: Mean, S.E, critical difference and coefficient of variation of quantitative traits of lentil germplasm lines.

Traits	Mean ±SEM	CD Value		CV %
		5%	1%	
Days to maturity	109.1 ± 0.57	1.57	2.08	0.90
Plant height	33.5 ± 0.63	1.76	2.31	3.26
Number of primary branches per plant	2.7 ± 0.12	0.33	0.44	7.68
Number of secondary branches per plant	5.2 ± 0.20	0.55	0.73	6.68
Total number of pods per plant	50.7 ±1.27	3.53	4.65	5.50
Number of effective pods per plant	37.1 ± 1.18	3.28	4.32	5.50
Number of seeds per plant	59.2 ±1.20	3.33	4.39	3.50
Number of Seeds per pod	1.6 ±0.005	0.14	0.19	5.70
100-seed weight	2.3 ±0.07	0.18	0.24	5.03
Seed yield per plant	1.2 ±0.11	0.30	0.40	15.88

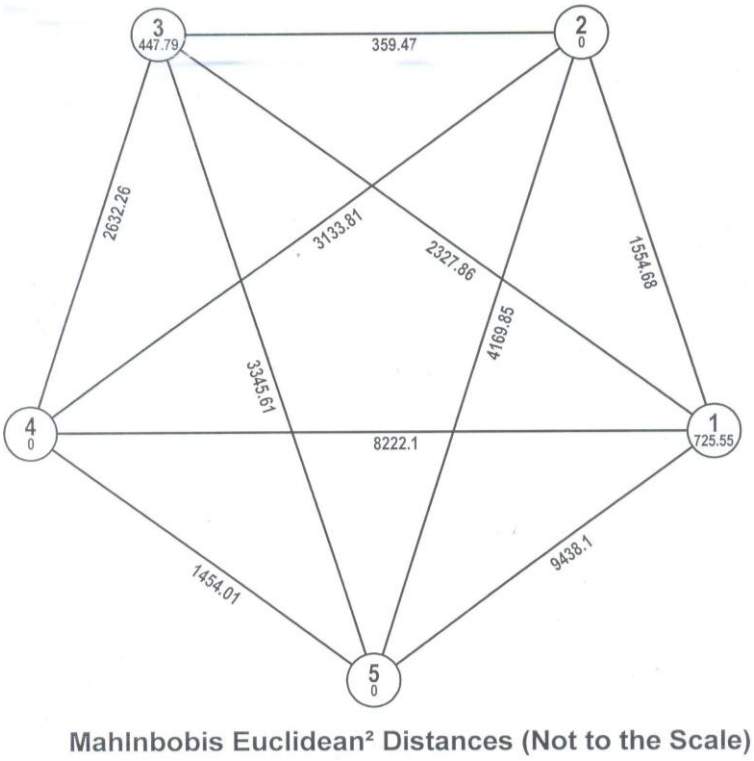

Fig 1: Diagram showing intra and inter cluster distances among V clusters

Table 2: Distribution of genotypes in different clusters.

		Number of genotype	% of Total
cluster	1	124	89.3
	2	1	0.72
	3	12	8.63
	4	1	0.72
	5	1	0.72
	Combined	139	100
Total	139	100 %	

Table 3: Average inter and intra cluster D^2 values for different clusters.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	**725.55**				
Cluster II	1554.68	**0.00**			
Cluster III	2327.86	359.47	**447.79**		
Cluster IV	8222.10	3133.81	2632.26	**0.00**	
Cluster V	9438.10	4169.85	3345.61	1454.01	**0.00**

Table 4: Cluster mean for yield and yield contributing traits of 139 lentil genotypes .

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Days to maturity	108.38	115.67	115.06	117.67	111.00
Plant height	33.08	32.30	38.29	33.60	35.47
Number of primary branches per plant	2.69	2.77	2.82	2.80	3.17
Number of secondary branches per plant	5.09	6.80	5.55	6.80	7.17
Total number of pods per plant	46.21	66.37	82.91	97.00	163.00
Number of Effective pods per plant	32.90	57.97	66.30	82.50	139.00
Number of seeds per plant	49.86	108.90	125.03	201.77	231.80
Number of seeds per pod	1.51	1.87	1.90	2.43	1.67
100-seed weight (g)	2.29	2.00	2.18	1.80	1.73
Seed yield per plant (g)	1.04	1.43	2.19	3.67	4.53

Table 5: Contribution (%) of different characters towards clustering.

Source	Times ranked 1st	Contribution %
Days to maturity	2413	25.16
Plant height (cm)	380	3.96
Number of primary branches	18	0.19
Number of secondary branches	141	1.47
Total number of pods/plant	1289	13.44
Number of Effective pods/plant	115	1.20
Number of seeds/plant	4509	47.01
Number of seeds/pod	35	0.36
100-seed weight (g)	555	5.79
Seed yield (g)	136	1.42

Table 6: Principal component analysis of lentil germplasm.

Trait	I Vector	II Vector	III Vector
Days to maturity	0.2568	**0.7924**	0.0489
Plant height	0.1156	**0.2660**	0.0496
Number of primary branches per plant	-0.0647	-0.2381	0.0201
Number of secondary branches per plant	0.0246	-0.0881	0.0904
Total number of pods per plant	0.4206	-0.1492	**0.7912**
Number of effective pods per plant	0.1401	-0.2123	0.1615
Number of seeds per plant	**0.7753**	-0.2247	-0.5109
Number of Seeds per pod	-0.0837	0.0662	0.2288
100-seed weight	-0.1232	-0.3345	0.0279
Seed yield per plant	-0.3101	0.0530	-0.1422

Conclusion

The results indicated that the germplasm lines studied had a considerable level of variability that could be exploited in future breeding programs. On the basis of cluster mean, intra and inter cluster distance and per se, cluster III, IV and V may be used for their desirable characters in breeding programme of lentil. Entries like, JLC 54, JLC 117, JLC 132, JLC 123, JLC 113 and JLC 35 were selected and progenies derived from intermating such diverse crosses are expected to show wide spectrum of genetic variability and a greater scope for isolating transgressive segregants in the advanced generations. Hence, these genotypes might be used in a multiple crossing programme to recover transgressive segregants.

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