

MORPHOLOGICAL CHARACTERIZATION AND GENETIC DIVERSITY IN LENTIL (*Lens culinaris* Medikus ssp. *culinaris*) GERMPLASM

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Abstract

Genetic divergence of 110 lentil germplasm with checks was assessed based on morphological traits using multivariate analysis. Mahalanobis generalized distance (D₂) analysis was used to group the lentil genotypes. Significant variations among lentil genotypes were observed in respect of days to 1st flowering, days to 50% flowering, days to maturity, plant height, and number of pods per peduncle, number of pods per plant, number of seeds per plant, 100 seed weight and yield per plant. Considering the mean values, the germplasm were grouped into ten clusters. The highest number of genotypes (17) was in cluster X and lowest (5) both in cluster II and IV. Cluster IV had the highest cluster mean for number of pods per plant (297.08), number of seeds per plant (594.16), 100 seed weight (1.44 g) and yield per plant (8.53 g). Among them, the highest inter-cluster distance was obtained between the cluster IV and I (24.61) followed by IV and III (22.33), while the lowest was between IX and II (1.63). The maximum value of inter-cluster distance indicated that genotypes belonging to cluster IV were far diverged from those of cluster I. The first female flower initiation was earlier in BD-3812 (49 days) in cluster I and cluster IV had highest grain yield per plant (8.53). BD-3807 produced significant maximum number of pods per plant (298.40) in cluster IV.

Keywords: Lentil, Morphological Characterization, Genetic Diversity, Germplasm

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Introduction

Lentil (*Lens culinaris* Medikus ssp. *culinaris*) is a major and important food legume in Bangladesh. In Bangladesh, all the indigenous landraces and cultivars are *microsperma* with red cotyledons. Lentil is the early domesticated among crops. It plays an important role in human, animal and soil health improvement. Nutritionally, lentil is very rich and complementary to any cereal crop including rice. It is versatile source of nutrients for man, animals and soil containing, on an average, 25.1% protein, 59% carbohydrate, 0.5% fat, 2.1% minerals and sufficient amount of vitamins, viz. vitamin A 16 IU; thiamine 0.23 mg and vitamin C 2.5 mg per gram lentil (Anonymous, 2003; Frederick *et al.*, 2006). Lentil fixes atmospheric nitrogen association with *Rhizobium* sp for this its cultivation improves soil nitrogen, carbon and organic matter balance in soil. Lentil ranks second in respect of area and production but consumer's preference its rank first among pulses in Bangladesh condition. Farmers are cultivated with sole crop or mixed crop with mustard in rabi season. Production of lentil has long lagged behind domestic demand in Bangladesh, where it is preferred pulse crop for human consumption. Therefore, it needs to increase its production with high yielding variety of lentil. Effort should

be made to develop such disease resistant varieties. Local germplasm of crop plants is an excellent source of economically useful plant characters (Pecetti *et al.*, 1996). The breeders must have a mean of choosing the accession most likely to possess the trait of interest. Quantitative traits provide an estimate of genetic diversity and various numerical taxonomic techniques have been successfully used to classify and measure the pattern of phenotypic diversity in the relationship of germplasm collections in a variety of crops by many scientists as in lentil (Ahmad *et al.*, 1997; Fratini *et al.*, 2007; Tullu *et al.*, 2008), Pea (Amurrio *et al.*, 1995) and Alfalfa (Smith *et al.*, 1991; Smith *et al.*, 1995; Warburton and Smith, 1993). Worldwide, lentil is grown on a total of 1.8 million hectares, of which 60% is in the South Asian region, which includes the lentil producing countries of Bangladesh, Burma, India, Nepal and Pakistan (Nazir *et al.*, 1994). However, lentil in general, does not respond to high inputs such as fertilizer and irrigation. Genetic diversity has been considered an important factor in any crop improvement program. However, the experiment of lentil was conducted to assess the genetic diversity of lentil genotypes in respect of agro-morphological traits.

Materials and Methods

The investigation was carried out at Regional Plant Genetic Resources Center, RARS, Bangladesh Agricultural Research Institute, Ishurdi, Pabna during the rabi season of 2010-11. The experiment was laid out non-replication. Unit plot size was 4m x 1m and seeds were sown in rows with spacing 40 cm. The seeds were sown on 30 October, 2010. The experiment involves two-hundred and twenty lentil germplasm and two varieties BARI Masur 4 and BARI Masur 6 were used as test genotypes. Fungicide, insecticide and other intercultural operations were done as and when necessary. Ten plants were sampled at random to study inter and intra-accession variation. Quantitative traits were recorded on 10 plants following IBPGR Descriptors (Anonymous, 1985). Morphological parameters of quantitative data were recorded on days to flowering, days to 50% flowering, plant height, number of pods per peduncle, number of pods per plant, number of seeds per pod, number of seeds per plant, 100 seed weight, yield per plant and different qualitative characteristics were recorded. Data were analyzed statistically by non-replication with computer and the means were separated. Genetic diversity was studied following Mahalanobis's generalized distance analysis (D2). Statistical analysis was done using the GENSTST 5 programme.

Results and Discussion

Qualitative characteristics

Qualitative characteristics of 110 genotypes are presented in Table 1. Variation between and within populations of crop species is useful for analyzing and monitoring germplasm during the maintenance phase and predicting potential genetic gain in a breeding programme (Hayward and Breese, 1993; Moore and Collins, 1983). For qualitative characteristics, a considerable level of variability was observed for growth habit, seedling stem pigmentation leaf pubescence, leaflet size, flower ground colour, seed coat colour, seed coat pattern and seed coat pattern colour, cotyledon colour and lodging susceptibility that could be exploited for developing future breeding material in lentil breeding programme (Table 1). Two classes were observed for stem colour, hairiness, tendril, pod pigmentation (anthocyanin), pod indehiscence, beak on the pod and cotyledon colour, whereas for other traits more than one class were observed, especially for seed traits that represents the world classification as reported by Erskine and Witcombe (1984). Muehlbauer and Slinkard (1981) reviewed the genetics of *Lens* and listed 12 genes, which account for morphological and seed variation in lentil. It was observed critically that seed traits in lentil are difficult to record that need standardization.

Table 1. Method for measuring and classifying of plant descriptors in 110 accessions of *lens culinaris* qualitative characteristics of lentil germplasm

Sl. No.	Plant characteristics	When and where measured	Classification
01	Seedling stem pigmentation	Observed at Seedling stage when plants were 2-3" high	Present (88), absent (22)
02	Leaf pubescence	Before maturity when plants were full grown	Absent (3), slight (88), dense (19)
03	Leaflet size	Observed on fully expanded leaves on the lower flowering nodes	Small (30), medium (28), large (52)
04	Tendril length	At the time of pod formation when plants were full grown	Rudimentary (35), prominent (75)
05	Flower ground colour	At 50% flowering. Ground colour of standard petal	Pink (12), white (98)
06	Pod pigmentation	Before maturity when pods were filled but not turned brown	Absent (54), present (56)
07	Pod shedding	Scored after or during harvesting a week after maturity	None (0), low (0), medium (0), High (0)
08	Pod dehiscence	At the time of maturity observed carefully to scored this trait up to one week after maturity	None (102), low (8), medium (0), high (0)
09	Ground colour of testa	To be observed on seed less than 3 months old	Green (2), grey (64), brown (18), black (14), pink (12)
10	Pattern of testa	To be observed on seed less than 3 months old	Dotted (77), spotted (17), marbled (16)
11	Colour	To be observed on seed less than 3 months old	Olive (12), grey (67), brown (18), black (13)
12	Pattern on testa	After harvesting but less than three months old	Yellow (22), orange/red (88)
13	Cotyledon colour	Scored at maturity	Low (32), medium (60), high (14), none (04)
14	Lodging susceptibility	Scored at maturity	Low (70), medium (32), high (4), none (4)
14	Pest and disease susceptibility	Before flowering when plants were full grown	Low (70), medium (32), high (4), none (4)

Quantitative characteristics

Yield and yield contributing characteristics

Yield and yield contributing characteristics of different lentil germplasm are presented in Table 2. Days to 1st flowering, days to 50% flowering, days to maturity, 100 seed weight and seed yield differed significantly among the entries. The first female flower initiation was noticed in BD-3812 (49 days). Maximum numbers of germplasm 104 were matured within 114-116 days where 6 germplasm matured within 117-118 days. Plant height varied from 35.80 cm to 48.60 cm, 64 germplasm were long (40.00-48.60 cm), 35 germplasm were medium (38.0-39.8 cm) and rest lines were dwarf (35.80-37.60 cm). Number of pods per plant varied from 96.0 to 325.0, 2 germplasm were highest number pods per plant (306.0-325.0), 52 germplasm were moderate number pods per plant (200.00-

298.40) and rest lines were lowest (96.0-196.0). Yield varied from 1.52 g per plant to 12.24 g per plant, where BD-3894 and BD-3902 were high yielding (11.76 -12.24 g/plant), 14 germplasm were moderate yielding (7.03- 9.58 g/plant) and rest germplasm were low yielding (1.52-6.91 g/plant). The highest seed yield (12.24 g/plant) was recorded from BD-3894 lentil germplasm and the lowest yield (1.52 g/plant) from BD-3835 lentil germplasm. Sultana *et al.* (2010) also reported that eight lentil accessions gave seed weight more than 3.1 g and hence could be utilized for the manipulation of this trait as high seed weight in any grain crop. Variability for these traits in lentil germplasm was also reported by Agrawal *et al.* (1976), Tiwari and Singh (1980), Malik *et al.* (1984) and Toklu *et al.* (2009). Singh and Singh (1993) confirmed the wide range of variation in agronomic characteristics of lentil germplasm, except for seeds per pod.

Table 2. Range, mean, SD and CV% of yield and yield contributing characteristics of 110 lentil germplasm

Characters	Range	Mean	SD	CV (%)
Days to 1st flowering	49.0-68.0	59.65	4.98	8.36
Days to 50% flowering	59.0-78.0	69.88	4.46	6.38
Days to maturity	114.0-118.0	115.24	0.98	0.85
Plant height(cm)	35.80-48.60	40.64	2.45	6.02
No. of pods/peduncle	1.0-3.0	1.87	0.38	20.60
No. of pods/plant	96.0-325.0	196.80	43.08	21.90
No. of seeds/plant	192.0-612.0	382.43	87.47	22.87
100 seed wt(g)	0.40-2.60	1.37	0.36	26.26
Yield per plant(g)	1.52-12.24	5.21	1.90	36.33

Genetic diversity in lentil germplasm

A considerable amount of genetic variability was observed and therefore diversity analysis was carried out through multivariate analysis.

The clustering pattern and distribution

Table 3. Distribution of 110 genotypes of lentil in 10 clusters

Clusters	No of genotypes	Genotypes
I	15	BD-3843, BD-3846, BD-3849, BD-3812, BD-3809, BD-3827, BD-3824, BD-3909, BD-3879, BD-3880, BD-3884, BD-3890, BD-3873, BD-3964, BD-3970
II	5	BD-3835, BD-3826, BD-3883, BD-3858, BD-3863,
III	11	BD-3856, BD-3853, BD-3852, BD-3839, BD-3840, BD-3805, BD-3829, BD-3874, BD-3892, BD-3867, BD-3966
IV	5	BD-3807, BD-3821, BD-3902, BD-3894, BD-3886
V	9	BD-3848, BD-3836, BD-3810, BD-3897, BD-3887, BD-3908, BD-3859, BD-3986, BD-3863
VI	10	BD-3844, BD-3834, BD-3804, BD-3820, BD-3901, BD-3877, BD-3907, BD-3869, BD-3866, BD-3871
VII	16	BD-3847, BD-3837, BD-3838, BD-3808, BD-3817, BD-3810, BD-3876, BD-3881, BD-3882, BD-3898, BD-3900, BD-3857, BD-3861, BD-3865, BD-3968
VIII	9	BD-3841, BD-3842, BD-3833, BD-3815, BD-3823, BD-3819, BD-3885, BD-3891, BD-3987
IX	13	BD-3845, BD-3850, BD-3854, BD-3851, BD-3831, BD-3822, BD-3818, BD-3830, BD-3906, BD-3895, BD-3878, BD-3899, BD-3889
X	17	BD-3832, BD-3811, BD-3806, BD-3855, BD-3825, BD-3828, BD-3905, BD-3896, BD-3875, BD-3888, BD-3893, BD-3872, BD-3860, BD-3868, BD-3870, BD-3985, BD-3988

Table 4. Means for quantitative characteristics for 10 clusters in lentil germplasm

No. of clusters	Days to 1st flowering	Days to 50% flowering	Days to maturity	Plant height (cm)	No. of pods/peduncle	No. of pods/plant	No. of seeds /pod	No. of seeds /plant	100 seed weight (g)	Yield/plant (g)
Cluster -1	60.60	70.53	115.07	39.64	1.93	135.83	1.95	258.08	1.36	3.48
Cluster -2	62.60	70.60	115.60	40.08	1.60	192.60	2.00	385.20	1.06	4.09
Cluster -3	59.91	71.18	115.73	41.78	2.18	152.47	1.98	290.31	1.41	4.09
Cluster -4	58.80	69.20	115.20	40.08	1.80	297.08	1.92	594.16	1.44	8.53
Cluster -5	59.67	69.33	115.11	41.36	1.89	213.44	1.87	421.78	1.40	5.91
Cluster -6	59.30	69.40	115.20	39.88	1.80	252.14	1.96	499.00	1.37	6.87
Cluster -7	59.38	69.87	115.37	39.38	1.94	174.51	1.94	335.16	1.37	4.59
Cluster -8	57.44	68.89	115.11	41.11	1.67	203.93	1.93	403.04	1.32	5.32
Cluster -9	59.92	69.54	115.08	40.34	1.85	189.89	1.94	364.48	1.30	4.74
Cluster -10	59.47	69.82	115.18	42.35	1.82	232.13	1.94	446.56	1.44	6.41

Non-hierarchical clustering

The covariance matrix gave non-hierarchical clustering among 110 genotypes and grouped them into ten clusters (Table 3). They coincided with the apparent grouping patterns performed by PCA. Cluster VII and X both contained the largest number of genotypes (sixteen), followed by clusters I, IX, III, V, VI, VIII, IV, and II. The genotypes of different geographic origin are accumulated in the same cluster indicating that the genotypes are not sharply diversified. Similar results were obtained by Alam *et al.* (2006) in Barley, Alam *et al.* (2011) in Lentil. These clusters lead to the highest cluster mean for maximum characters (Table 4). Among the ten characters, the highest mean values for four characters, viz. number of pods per plant (297.08), number of seeds per plant (594.16), 100 seed weight (1.41 g), and yield per plant (8.53 g) were found in cluster IV. Cluster IV had only five genotypes, viz. BD-3807, BD-3821, BD-3902, BD-3894 and BD-3886. Cluster X with sixteen genotypes was able to lead only for two traits in respect of cluster means of six characters. The highest cluster mean was recorded for 100-seed weight (1.41 g). Cluster

VI, X and V was moderate yielding associated with desired characteristics like size and early maturity.

Canonical Variate Analysis

Average inter-cluster D2 values among the ten clusters are presented in Table 5. The highest inter-cluster distance was obtained between the cluster IV and I (24.61) followed by IV and III (22.33), while the lowest was between IX and II (1.63). The maximum value of inter-cluster distance indicated that genotypes belonging to cluster IV were far diverged from those of cluster I. Similarly, the highest inter-cluster values between cluster IV and III indicated that the genotypes between each pair of clusters were more diverged. Sultana *et al.* (2010) also reported that the inter-cluster distance among the accessions revealed that the cluster V consisting of five accessions was obviously very much different from all the other clusters with a genetic distance ranging from 1.99 (Cluster VIII) to 2.85 (Cluster X).

Table 5. Average inter and intra (bold) cluster distance (D2) for the 110 lentil germplasm obtained on the basis of the morphological characteristics

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	0.416									
II	9.30	0.717								
III	2.68	7.25	0.456							
IV	24.61	15.35	22.33	0.646						
V	12.04	2.94	9.78	12.570	0.438					
VI	17.65	8.40	15.40	6.956	5.620	0.485				
VII	5.73	3.60	3.67	18.876	6.318	11.920	0.415			
VIII	10.71	1.74	8.45	13.905	1.336	6.954	4.983	0.425		
IX	7.87	1.63	5.68	16.740	4.176	9.784	2.142	2.841	0.442	
X	13.96	4.97	11.60	10.744	2.033	3.899	8.260	3.311	6.124	0.351

Principal Coordinate Analysis (PCO)

The results obtained from principal coordinate analysis showed that the highest inter-genotypic distance was between genotypes BD-3894 and BD-3970 (1.964), followed by BD-3849 and BD-3894 (1.868), and the lowest (0.083) between genotypes BD-3840 and BD-3805 as well as

between BD-3909 and BD-3964 (0.102) (Table 5). The difference between the highest and lowest inter-genotypic distance indicated that moderate variability among 110 germplasm of grass pea. The highest intra-cluster distance was recorded in cluster II (0.717) containing five genotypes viz. BD-3835, BD-3826, BD-3883, BD-3858 and BD-

3863 (Table 6). The lowest intra-cluster distance was in cluster X (0.351) and containing also seventeen genotypes genotypes, viz. BD-3832, BD-3811, BD-3806, BD-3855, BD-3825, BD-3828, BD-3905, BD-3896, BD-3875, BD-3888, BD-3893, BD-3872, BD-3860, BD-3868, BD-

3870, BD-3985 and BD-3988. Similar types of research were conducted by Malik *et al.* (1984) who reported high genetic variance in cultivated lentil.

Table 6. Five highest and lowest inter-genotypic distances among one hundred seven genotypes of grasspea

Genotypic combination		Genotypic combination	
A. Five lowest inter-genotypic distances	Distance	B. Five highest inter-genotypic distances	Distance
BD-3851 – BD-3863	0.130	BD-3894–BD-3970	1.964
BD-3893– BD-3988	0.123	BD-3849–BD-3894	1.868
BD-3875– BD-3888	0.118	BD-3894– BD-3966	1.861
BD-3909– BD-3964	0.102	BD-3821– BD-3970	1.844
BD-3840–BD-3805	0.083	BD-3849– BD-3902	1.810

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) helps to assessment of diversity in multivariate scales. Principal Component Analysis was carried out with 110 genotypes of lentil. The first five Eigen values for five principal coordination axes of genotypes accounted for 83.48 % variation while only first two principal coordination axes of genotypes accounted for 46.77 % of total variation among the ten characteristics (Table 7).

Bozokalfa *et al.* (2009) also reported that the first six axes accounted for 54.29% of the variability among the 48 accessions and their lines. Alam *et al.* (2011) also reported that the first three Eigen values for three principal coordination axes of lentil genotypes accounted for 78.1% variation. The first two principal axes accounted for 61.2% of total variation among six characters.

Table 7. Eigen values and percentage of variation for corresponding 10 component characteristics of 110 lentil germplasm

Principal component axis	Eigen values	% of total variation accounted for	Cumulative percent
Days to 1st flowering	2.829	28.29	28.29
Days to 50% flowering	1.848	18.48	46.77
Days to maturity	1.389	13.89	60.66
Plant height(cm)	1.272	12.72	73.38
No. of pods/peduncle	1.010	10.10	83.48
No. of pods/plant	0.791	7.91	91.39
No. of seeds/pod	0.685	6.85	98.24
No. of seeds/plant	0.134	1.34	99.58
100 seed weight(g)	0.029	0.29	99.87
Yield per plant(g)	0.014	0.14	100.0

Contribution of characteristics towards the divergence of genotypes

The values of vector 1 and vector 2 are presented in Table 8. The value of vector 1 obtained from PCA for days to first flowering, days to 50% flowering, days to maturity, number of pods per peduncle and number of seeds per pod suggests it was the major characteristics that contributed to the genetic divergence. In vector 2 also obtained from PCA for days to 1st flowering (0.631), days to 50% flowering (0.622), days to maturity (0.115), Plant height (0.122), number of pods per peduncle(0.038), number of pods per plant (0.270), 100-seed weight (0.269) and yield per plant (0.080) showed their important role toward

genetic divergence. Both vector 1 and vector 2 revealed that vector 1 had positive values for days to first flowering, days to 50% flowering, days to maturity, number of pods per peduncle and number of seeds per pod and vector 2 had positive values for days to 1st flowering, days to 50% flowering, days to maturity, Plant height (cm), number of pods per plant, 100 seed weight (g) and yield per plant (g) are indicating highest contribution of these traits towards the divergence among 110 genotypes of lentil. Negative values in both vectors had lower contribution towards the divergence. Similar results were obtained by Alam *et al.* (2011) in lentil.

Table 8. Relative contributions of the 10 characteristics to the total divergence in Lentil germplasm

Characteristics	Vector 1	Vector 2
Days to 1st flowering	0.230	0.631
Days to 50% flowering	0.213	0.622
Days to maturity	0.070	0.115
Plant height(cm)	-0.081	0.122
No. of pods/peduncle	0.115	0.038
No. of pods/plant	-0.509	0.270
No. of seeds/pod	0.050	-0.098
No. of seeds/plant	-0.272	-0.150
100 seed weight(g)	-0.509	0.269
Yield per plant(g)	-0.529	0.080

Conclusion

Days to 1st flowering, days to 50% flowering, days to maturity, number of pods per peduncle, number of pods per plant, number of seeds per pod, 100 seed weight (g) and yield per plant (g) had highest contribution towards divergence among 10 characteristics for 110 lentil germplasm. Based on analysis the germplasm grouped into ten clusters. From morphological study, the highest inter-cluster distance was obtained between the cluster IV and I (24.61) followed by IV and III (22.33). The intra-cluster distance was highest (0.717) in cluster II and lowest (0.315) in cluster X. Considering yield performance, cluster distance and cluster mean, the genotypes BD-3894, BD-3821 and BD-3902 from cluster IV and BD-3804, BD-3902 and BD-3869 from cluster III may be considered better genotypes for recombination breeding due to their larger divergence.

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