MORPHOLOGICAL CHARACTERIZATION AND GENETIC DIVERSITY IN LENTIL (*LENS CULINARIS MEDIKUS SSP. CULINARIS*) GERMPLASM

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PURPOSE
LENTIL is a major and important food legume. It plays an important role in human, animal and social health improvement. Nutritionally, lentil is very rich and complementary to any cereal crops including rice.

Lentil fixes atmospheric nitrogen association with Rhizobium sp, for this it's cultivation improves soil nitrogen, carbon and organic matter balance in soil. Genetic diversity has been considered an important factor in any crop improvement programme. However, the experiment of lentil was conducted to assess the genetic diversity of lentil genotypes in respect of agro-morphological traits.

Design/Methodology/Implications: The investigation was carried out at regional plant genetic resources centre, RARS, Bangladesh Agricultural Institute, Iishurdi, Pabna, during the Rabi season of 2010-11. The experiment involved 220 lentil Germplasm two varieties of BARI Masur, six were used as test genotype.

Data was analysed statistically by non-replication with computer and the mean were separated. Genetic diversity was studied following Mahalanobis's generalized distance analysis(D2). Statistical analysis were done using the GENSTST 5 programme. The experiment involved 220 lentil germplasm and two varieties of BARI Masur 4 and Bari Masur 6 were used as test genotype.

Findings: Significant variations among lentil genotypes were observed in respect of days to 1st flowering, days to 50% flowering, days to maturity, plant height and numbers of pods per peduncles, numbers of pods per plant, numbers of seeds per plant, 100 seeds weight and yield per plant. Considering the mean value, the germplasms were grouped into 10 clusters. The highst number of genotype (17) was in cluster X and lowest (5) both in cluster II & IVB. Cluster IV had the highest cluster mean for number of pods per plant (297.08), number of seeds per plant (594.16), 100 seeds weight (1.44 gm) and yield per plant (8.53g). Among them, the highest inter cluster distance was obtained between the cluster IV &I (24.61) followed by IV &III (22.33), while the lowest was between IX&II (1.63). The maximum value of intercluster distance indicated that the 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

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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.

Research Limitation/Implications: The investigation was carried out at RARS, Bangladesh Agricultural Research Institute, Ishurdi, Pubna during the Rabi season 2010-11. Due to time and seasonal constraints the experiment was laid only on unit plot size of 4m x 1m, and seeds were sown in rows with spacing 40 cm. The research will help in increasing the cereal production during Rabi season.

Key words: Lentil, Morphological Characterization, Genetic Diversity, Germplasm.

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 micro-sperma 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 a versatile source of nutrition 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 and Muehlbauer et al., 2006). Lentil fixes atmospheric nitrogen association with Rhizobium sp due to this its cultivation improves soil nitrogen, carbon and organic matter balance in soil. Lentil ranks second in respect of area and production but consumers' prefer its rank first among pulses in Bangladesh condition. Farmers are cultivating 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 a 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 posses 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 & 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 on 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 No. 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 & Breese, 1993; Moore & Collins, 1983). For qualitative characteristics, a considerable level of variability was observed for in 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 No. 1). Two classes were observed for stem colour, hairiness, tendril, pod pigmentation (anthocianin), pod indehiscence, beak on the pod and cotyledon colour, whereas for other traits more than one classes were observed, especially for seed traits that represents the world classification as reported by Erskine & Witcombe (1984). Muehlbauer & 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 No. 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
1.	Seedling stem pigmentation	Oberved at Seedling stage when plants were 2-3" high	Present (88), absent (22)
2.	Leaf pubescence	Before maturity when plants were full grown	Absent (3), slight (88), dense (19)
3.	Leaflet size	Observed on fully expanded leaves on the lower flowering nodes	Small (30), medium (28), large (52)
4.	Tendril length	At the time of pod formation when plants were full grown	Rudimentary (35), prominent (75)
5.	Flower ground colour	At 50% flowering. Ground colour of standard petal	Pink (12), white (98)
6.	Pod pigmentation	Before maturity when pods were filled but not turned brown	Absent (54), present (56)
7.	Pod shedding	Scored after or during harvesting a week after maturity	None (0), low (0), medium (0), High(0)
8.	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)
9.	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.	ColourPattern on testa	To be observed on seed less than 3 months old	Olive (12), grey (67), brown (18), black (13)
12.	Cotyledon colour	After harvesting but less than 3 months old	Yellow (22), orange/red (88)
13.	Lodging susceptibility	Scored at maturity	Low (32), medium (60), high (14), none (04)
14.	Pest and disease susceptibility	Before flowering when plants were full grown	Low (70), medium (32), high (4), none (4)

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Quantitative Characteristics

Yield and Yield Contributing Characteristics

Yield and yield contributing characteristics of different lentil germplasm are presented in Table No. 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 whereas 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 & Singh (1980), Malik et al. (1984) and Toklu et al. (2009). Singh & Singh (1993) confirmed the wide range of variation in agronomic characteristics of lentil germplam, except for seeds per pod.

Table No. 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 No. 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 No. 4: Means for Quantitative Characteristics for 10 Clusters in Lentil Germplasm

No. of Clusters	Days to 1st Flow- ering	Days to 50% Flow- ering	Days to Matu- rity	Plant Height (cm)	No. of Pods/ Pedu- ncle	No. of Pods/ Plant	No. of Seeds Pod	Seeds	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 No. 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 No. 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 (1.41g) and yield per plant (8.53g) 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 No. 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 No. 5: Average Inter and Intra (bold) Cluster Distance (D2) for the 110 Lentil Germplasm Obtained on the Basis of the Morphological Characteristics

Clusters	Ι	П	Ш	IV	V	VI	VII	VIII	IX	X
I	0.416									
Π	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 No. 6). The difference between the highest and lowest inter-genotypic distance indicated that moderate variability among 110 germplasm of grasspea. 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 No. 5). The lowest intra-cluster distance was in cluster X (0.351) and containing also seventeen genotypes geno-types, viz. BD-3832, BD-3811, BD-3806, BD-3855, BD-3825, BD-3828, BD-3905, 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 No. 6: Five Highest and Lowest Inter-genotypic Distances Among One Hundred Seven Genotypes of Grass Pea

Genotypic Combination	Distance	Genotypic Combination	Distance
A.Five lowest inter-genotypic distances		B.Five highest inter-genotypic distances	
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 No. 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 No. 7: Eigen Values and Percentage of Variation for Corresponding 10 Component Characteristics of 110 Lentil Germplasm

Principal Component	Eigen Values Axis	% 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 No. 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), 100seed 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, 100seed 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 No. 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-3821and 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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