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Characterization of lentil (*Lens culinaris* M.) germplasm through phenotypic marker

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Abstract

An experiment was carried out to evaluate the performance of 22 lentil (*Lens culinaris* M.) genotypes for their yield and yield contributing characters at phenotypic level, relationship and diversity among the individuals and characterize the germplasm through phenotypic markers. ANOVA table revealed that significant variation was found among the genotypes for the characters plant height, days to maturity, number of primary branches plant⁻¹, number of secondary branches plant⁻¹, height of the lowest pod and 100-seed weight. Yield plant⁻¹ was positively correlated with pods plant⁻¹, seeds pod⁻¹, seeds plant⁻¹ and 100-seed weight. Considering yield plant⁻¹ LM-123-7 was the best followed by LM-132-7, ICARDA-2348 and LM-13-1. Days to flowering, days of maturity and 100-seed weight showed high heritability. Path analysis revealed that pods plant⁻¹ and 100-seed weight had positive direct effects on yield plant⁻¹. The genotypes were grouped into five clusters based on Euclidean distance following Ward's method and the highest intra-cluster distance was found in cluster IV and inter-cluster distance between the cluster IV and cluster V. Study of selection indices through discriminate functions observed that LM-123-7 ranked as the best followed by LM-132-7, LM-21-6, LM-24-3 and LM-14-2 and suggests that the highest scoring genotypes might be recommended for farmer's cultivation for better yield.

Keywords: Lentil genotypes, Coefficients of correlation, Path analysis, Clustering, Selection index

Introduction

Lentil (Lens culinaris ssp. culinaris) is a diploid (2n=2x=14), autogamous species and is one of the oldest crops in the world, which originated in the Near East (Zohary, 1972). The cultivated lentil belongs to the family Leguminosae, sub-family Papilionaceae. The genus Lens comprises seven taxa within four species including the cultivated type, Lens culinaris spp. culinaris (Ferguson and Erskine, 2001). Cultivated lentil includes two varietal types: small-seeded microsperma and large-seeded macrosperma. The major lentil producing regions are Asia (58 percent of the area) and the West Asia-North Africa region (37 percent of the acreage of developing countries). In 2010, the total area used in the whole world was 4189502 ha where the total production was 4585439 tons. In Bangladesh the land area used for lentil cultivation was 77287 ha and the production of lentil was about 71100 tons (FAOSTAT, 2010). The largest and most representative collection of lentil landraces is maintained by the International Center for Agricultural Research in the Dry Areas (ICARDA) (3351 accessions), and large collections are also held in the USA (2807 accessions), Russia (2857 accessions), Australia (2847 accessions) and Turkey (1435 accessions); (Ford et al., 2007). Several of the lentil landraces that have exhibited significantly superior root and shoot traits and yield were considered to be valuable germplasms for breeding a drought-tolerant cultivar (Sarker et al., 2005). In Bangladesh, the lentil landraces must be characterized for use of the future lentil breeding program. So, this experiment is conducted to evaluate the performance of 22 lentil (Lens culinaris M.) genotypes by phenotypic markers and to determine the relationship and diversity among the individuals.

Materials and Methods

The experiment was conducted at the experimental farm of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, during November 2011 to March 2012. Geographically the experimental area is located at 24°75 N latitude and 90°5 E longitudes at the elevation of 18 m above the sea level. The field was medium high land. The soil of the experimental site belonging under the agro-ecological zone of Old Brahmaputra Floodplain (AEZ-9). The experimental field area was under sub-tropical climates characterized by heavy rainfall during the month of April to September and scanty rainfall during October

to March. The seeds of sixteen advanced lentil mutants (i.e. LM-123-7, LM-132-7, LM-28-2, LM-14-2, LM-21-6, LM-24-3, LM-37-8, LM-48-1, LM-99-4, LM-95-3, LM-13-1, LM-156-1, LM-185-2, LM-20-3, LM-67-5 and LM-75-4) and six ICARDA lines (i.e. ICARDA 2348, ICARDA 38211, ICARDA 23136, ICARDA 23121, ICARDA 23105 and ICARDA 23128) were collected from Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh. The experiment was laid out in a Randomized Complete Block Design (RCBD) with five replications. The experimental field was divided into five blocks each representing one replication. Each block was then sub-divided in to twenty two plots. The sixteen lentil mutants and six ICARDA lines was placed in each plot. The size of the unit plot was 2 m X 1.2 m. Row to row and plant to plant distances were 30 cm and 5 cm, respectively in each plot. The growth, yield and yield-contributing characters plant height (cm), number of primary branches plant 1, number of secondary branches plant 1, days to flowering, days to maturity, height of the lowest pod (cm), number of pods plant 1, seeds pod 1, seeds plant 1, 100-seed weight and yield plant 1 were recorded for phenotypic characterization. The statistical analysis was done by plant breeding statistical program (PLBSTAT, Version 2N, Utz, 2007). Estimation of heritability, estimation of correlation coefficients, estimation of selection index were done as per objectives.

Results and Discussion

The performance of different lentil genotypes for grain yield and different yield contributing characters were evaluated and there were significant variations among the genotypes for the characters plant height, days to maturity, number of primary branches plant¹, number of secondary branches plant¹, height of the lowest pod and 100-seed weight (Table 1).

Items	d. f.	Plant height (cm)	Days to flowering	Days of maturity	No. of primary branches plant-1	No. of secondary branches plant ⁻¹	Height of the lowest pod (cm)	No. of pod plant-1	No. of seeds pod ⁻¹	No. of seeds plant ⁻¹	100 seed weight (g)	Yield plant ⁻¹ (g)
Genotypes	21	63.72**	210.69**	34.95**	6.50**	41.31*	26.49**	9636	0.07	24642	0.59**	12.96
Replication	4	47.57	15.15	9.58	2.47	61.24*	7.70	3501	0.07	20626	0.03	10.20
Error	84	23.10	19.14	7.34	2.73	24.51	7.56	7143	0.04	18287	0.10	9.03

^{*} and ** indicates significant at 0.05 and 0.01 probability, respectively

In Table 2 it was found that the genotype ICARDA 23128 was the tallest plant and the genotype LM-28-2 was the shorter one. LM-24-3 was the earlier flowering genotype and the genotypes, LM-185-2, ICARDA-23105, LM-67-5 took the highest days to maturity (110 days) and the LM-37-8 was an early maturing one (102 days) this result was in full agreement with Dutta (2001). The genotype ICARDA-23136 had highest primary branches and the genotype ICARDA-23128 had highest secondary branches. The genotypes, ICARDA-38211 and ICARDA-23105 were maintained maximum height of the lowest pod then other genotypes. The highest number of pods plant was recorded in ICARDA- 2348 and the lowest was recorded in ICARDA-23121. The highest number of seeds pod was recorded in LM-13-1 and lowest number was found in ICARDA-23136. The lowest seeds plant was found in ICARDA-23136 and the highest was found in ICARDA- 2348 followed by ICARDA-23128. The highest 100-seed weight was recorded in LM-24-3 and the lowest was found in LM-37-8. The highest yield plant was recorded in LM-123-7 and the lowest in ICARDA-23136. Among the 11 characters, 50% flowering, days of maturity and 100-Seed weight were high heritable and plant height, number of primary branches plant number of secondary branches plant heritable and number of seeds pod plant were medium heritable and number of pod plant number of seeds plant were low heritable (Table 3).

Gupta *et al*. 199

Table 2. Mean performance of 22 genotypes based on different morphological traits related to yield

Genotypes	Plant height	Days to	Days of	No. of primary	No. of secondary	Height of the
,,	(cm)	flowering	maturity	branches plant-1	branches plant-1	lowest pod (cm)
LM-123-7	27.10 c-f	54.40 f-i	107 a-f	4.20 bc	12.80 a-c	5.80 b
LM-132-7	31.60 a-e	51.80 hi	106 b-f	3.80 bc	10.80 bc	6.68 b
LM-28-2	23.60 f	56.00 d-i	106 b-f	3.80 bc	8.00 c	5.80 b
LM-14-2	28.20 b-f	51.40 i	106 b-f	3.80 bc	7.80 c	5.60 b
LM-21-6	25.00 ef	53.20 g-i	107 a-f	3.60 bc	11.40 bc	6.20 b
LM-24-3	26.20 d-f	43.80 j	102 gh	4.60 bc	9.80 bc	6.00 b
LM-37-8	34.70 ab	51.80 hi	102 h	3.20 c	9.40 bc	7.60 b
LM-48-1	29.40 a-f	57.20 d-i	105 d-h	4.40 bc	10.20 bc	6.40 b
LM-99-4	26.80 c-f	56.00 d-i	105 d-h	4.20 bc	11.40 bc	7.60 b
LM-95-3	24.40 ef	61.60 b-e	103 f-h	3.20 c	9.00 c	8.20 b
LM-13-1	27.60 b-f	57.80 d-i	104 e-h	5.60 bc	13.80 a-c	6.20 b
LM-156-1	27.80 b-f	60.20 c-f	108 a-d	5.40 bc	12.80 a-c	6.60 b
LM-185-2	26.60 d-f	62.40 b-d	110 a	5.00 bc	11.60 bc	6.44 b
LM-20-3	29.60 a-f	55.80 e-i	108 a-d	5.40 bc	11.80 bc	6.80 b
LM-67-5	31.20 a-e	58.40 d-g	110 ab	5.80 b	11.80 bc	6.30 b
LM-75-4	27.40 b-f	60.80 c-f	109 a-c	4.20 bc	9.00 c	6.20 b
ICARDA 2348	32.80 a-d	66.20 a-c	109 a-d	5.00 bc	11.60 bc	9.10 b
ICARDA 38211	34.00 a-c	67.40 ab	110 ab	5.60 bc	14.40 a-c	14.20 a
ICARDA 23136	29.40 b-f	67.20 ab	107 а-е	8.20 a	16.80 ab	9.40 b
ICARDA 23121	30.20 a-f	65.80 a-c	108 a-d	5.40 bc	14.80 a-c	9.40 b
ICARDA 23105	34.60ab	58.20 d-h	110 ab	5.00 bc	13.60 a-c	13.20 a
ICARDA 23128	36.60a	71.20a	108 a-d	6.00 b	19.80 a	8.80 b
CV (%)	16.40	7.47	2.54	17.52	14.51	13.66
Maximum	36.6	71.2	110	8.2	19.8	14.2
Minimum	23.6	43.8	102	3.2	7.8	5.6
Mean	29.31	58.57	106.78	4.79	11.93	7.66
LSD (0.05)	6.04	5.50	3.41	2.08	6.23	3.46

Table 2. Contd.

Genotypes	No. of pod plant ⁻¹	No. of seed pod ⁻¹	No. of seed plant ⁻¹	100 seed weight (g)	Yield palnt⁻¹
LM-123-7	201 a-c	1.57 a-c	308 a-c	2.63 ab	7.99 a
LM-132-7	179 a-c	1.55 a-c	301 a-c	2.46 a-d	7.68 ab
LM-28-2	121 bc	1.58 a-c	188 bc	2.09 c-g	4.03 a-c
LM-14-2	152 bc	1.37 a-d	211 bc	2.55 a-c	5.52 a-c
LM-21-6	154 bc	1.42 a-d	221 a-c	2.37 b-d	5.39 a-c
LM-24-3	155 bc	1.54 a-c	239 a-c	2.85 a	6.90 a-c
LM-37-8	122 bc	1.61 a-c	199 bc	1.54 h	3.16 bc
LM-48-1	124 bc	1.58 ac	193 bc	2.27 b-e	4.03 a-c
LM-99-4	154 bc	1.54 a-c	249 a-c	2.44 a-d	6.11 a-c
LM-95-3	149 bc	1.41 a-d	190 bc	2.41 a-d	4.65 a-c
LM-13-1	192 a-c	1.68 a	322 a-c	2.24b-f	7.27 ab
LM-156-1	175 a-c	1.51 a-d	261 a-c	1.78 b-h	4.88 a-c
LM-185-2	158 bc	1.49 a-d	247 a-c	2.17 b-f	5.56 a-c
LM-20-3	142 bc	1.35 b-d	204 bc	1.79 f-h	3.50 a-c
LM-67-5	166 a-c	1.31 cd	221 a-c	2.30 b-e	5.18 a-c
LM-75-4	116 bc	1.55 a-c	194 bc	2.01 d-g	4.28 a-c
ICARDA 2348	288 a	1.46 a-d	418 a	1.85 e-h	7.65 ab
ICARDA 38211	126 bc	1.36 a-d	170 c	2.23 b-f	3.84 a-c
ICARDA 23136	120 bc	1.21d	149 c	1.68 gh	2.52 c
ICARDA 23121	105 c	1.63 ab	173 c	2.01 d-g	3.24 bc
ICARDA 23105	119 bc	1.58 a-c	181 bc	2.17b c-f	4.00 a-c
ICARDA 23128	241 ab	1.55 a-c	384 ab	1.68 gh	6.13 a-c
CV (%)	14.82	15.90	5.77	6.95	8.25
Maximum	288	1.68	418	2.85	7.99
Minimum	104	1.21	149	1.54	2.52
Mean	157.17	1.49	237.44	2.16	5.16
LSD (0.05)	106.30	0.26	170.10	0.40	3.78

Table 3. Heritability (h²b) of different traits of 22 lentil genotypes

Traits	Heritability, h ² b (%)
Plant height	73.40
Days to 50% flowering	91.67
Days to maturity	82.65
Number of primary branches plant ⁻¹	70.40
Number of secondary branches plant ⁻¹	62.76
Height of the lowest pod	77.79
Number of pod plant ⁻¹	57.43
Number of seeds pod ⁻¹	62.32
Number of seeds plant ⁻¹	57.40
100- Seed weight	85.12
Yield plant ⁻¹	58.93

Relationship between physiological and yield contributing characters was studied through analysis of correlation between them. Grain yield plant was positively correlated with pod plant, seed pod, seed plant 1 and 100 seed weight. Among them 100 seed weight was significantly correlated with grain yield plant suggesting that genotypes with high partitioning efficiency gave increase in grain yield plant plant (Table 4). The path coefficient analysis (Table 5) was performed using correlation coefficient to determine direct and indirect influence considering eight characters viz. plant height, days to flowering, days to maturity, number of primary branches plant⁻¹, number of secondary branches plant⁻¹, number of pods plant⁻¹, number of seeds pod⁻¹ and 100-seed weight to yield plant⁻¹. Days to maturity, number of primary branches plant⁻¹, number of secondary branches plant⁻¹, pods plant⁻¹, seeds pod⁻¹ and 100-seed weight had positive direct effects on grain yield plant⁻¹ which is agreement with the findings of Rasheed *et al.* (2008) and Younis et al. (2008). Among the characters pods plant and 100-seed weight had high positive direct effects on grain yield plant⁻¹. Plant height, and days to flowering showed negative direct effects on grain yield plant¹. The highest direct effect of number of pod plant¹ gave a significant positive correlation inducing that among all the traits under study these trait contributed maximum for grain yield. The residual effect was 0.27 indicating that the eight characters contributed 73 percent of variability in grain yield plant 1 studied in path analysis. The present study suggested that plant height, days to flowering, days to maturity, number of primary branches plant⁻¹, number of secondary branches plant⁻¹, pods plant⁻¹, and seeds pod⁻¹, 100-seed weight should be included owing to importance in selecting the genotypes for higher grain yield in lentil. Based on D²-value, the genotypes were grouped into five clusters viz. I, II, III, IV and V (Table 6).

Table 4. Coefficients of correlation among different yield components of 22 lentil genotypes

Traits	Plant height (cm)	Days to 50% flowering	Days of maturity	Number of primary branches plant-1	Number of secondary branches plant-1	Height of the lowest pod (cm)	Number of pod plant-1	Number of seeds pod-1	Number of seeds plant-1	100- Seed weight (g)
Days to 50% flowering	0.388									
Days of maturity	0.308	0.580**								
Number of primary branches plant-1	0.326	0.590**	0.454*							
Number of secondary branches plant-1	0.532*	0.672**	0.402	0.769**						
Height of the lowest pod (cm)	0.612**	0.551**	0.371	0.359	0.497*					
Number of pods plant-1	0.219	0.196	0.098	0.079	0.267	-0.127				
Number of seeds pod-1	0.018	-0.207	-0.326	-0.412	-0.077	-0.149	0.054			
Number of seeds plant-1	0.23	0.13	0.033	0.011	0.267	-0.187	0.960**	0.291		
100- Seed weight (g)	-0.480*	-0.580**	-0.263	-0.413	-0.404	-0.257	-0.026	0.071	-0.024	
Yield plant-1(g)	-0.109	-0.290	-0.129	-0.227	-0.037	-0.35	0.772**	0.289	0.820**	0.524*

^{*} and ** indicates significant at 0.05 and 0.01 probability, respectively

Gupta et al. 201

Table 5. Partitioning of phenotypic correlations into direct and indirect effects of eight important characters by path analysis

Characters	Plant	Days to	Days of	Number of	Number of	Number	Number of	100-Seed	Correlation to
	height	50%	maturity	primary	secondary	of pod	seeds pod-1	weight	yield plant-1
		flowering		branches	branches	plant-1			
		_		plant-1	plant-1				
Plant height	-0.070	-0.027	-0.022	-0.023	-0.037	-0.015	-0.001	0.033	-0.109
Days to 50% flowering	-0.104	-0.267	-0.155	-0.158	-0.179	-0.053	0.056	0.155	-0.290
Days of maturity	0.031	0.057	0.099	0.044	0.040	0.010	-0.031	-0.026	-0.129
Number of primary branches plant-1	2.182	0.039	0.030	0.066	0.051	0.005	-0.027	-0.027	-0.227
Number of secondary branches plant-1	0.027	0.034	0.020	0.039	0.050	0.014	-0.004	-0.020	-0.037
Number of pod plant-1	0.178	0.162	0.081	0.065	0.219	0.811	0.041	0.024	0.772**
Number of seeds pod-1	0.005	-0.048	076	-0.094	-0.018	0.011	0.230	0.016	0.289
100- Seed weight	-0.198	239	107	-0.169	-0.165	-0.012	0.029	0.413	0.524*
Residual effect = 0.27									

^{*} and ** indicates significant at 0.05 and 0.01 probability, respectively. Bold figures indicate the direct effect

Table 6. Clustering pattern of 22 genotypes of based on Euclidean distance following Ward's method and the member present in each respective cluster

Cluster number	Number of genotypes	Percent	Name of genotypes
I	4	18.18	LM-123-7, LM-132-7, LM-24-3 and LM-13-1
II	8	36.36	LM-28-2, LM-14-2, LM-21-6, LM-37-8, LM-48-
			1, LM-99-4, LM-95-3 and LM-75-4
III	4	18.18	LM-156-1, LM-185-2, LM-20-3 and LM-67-5
IV	2	9.09	ICARDA 2348 and ICARDA 23128
V	4	18.18	ICARDA 38211, ICARDA 23136, ICARDA
			23121 and ICARDA 23105

Table 7. Average intra and inter-cluster D² and D values of five clusters

Cluster	ı	II	III	IV	V
I	<u>16953</u>	53104	19199	167623	106170
	(130)	(230)	(139)	(409)	(326)
II		12789	18151	373299	19249
		(113)	(135)	(611)	(139)
III			<u>12708</u>	249447	51891
			(113)	(499)	(228)
IV				<u>48091</u>	496167
				(219)	(704)
V					<u>10621</u>
					(103)

Cluster I, III and V had same number of genotypes i.e. four and cluster II contained eight genotypes and cluster IV contained only two genotypes. The highest intra cluster distance was found in cluster IV. The maximum inter cluster distance was observed between genotypes of cluster IV and V followed by clusters II and IV (Table 7 and Fig.1). Thus, hybridization among genotypes drawn from these widely divergent clusters with high yield potential would likely to produce heterotic combinations and wide variability in segregating generations.

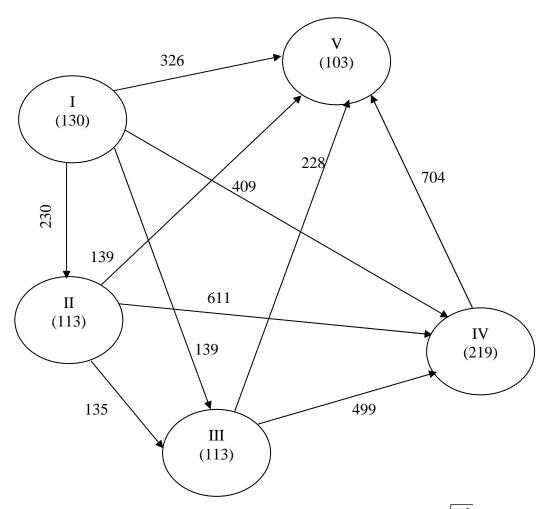


Fig. 1. Cluster diagram showing the average intra and inter cluster distances (D = $\sqrt{D^2}$ values) of 22 lentil genotypes. The values along the lines inter cluster distances and the values within the circle indicate intra cluster distances

Dendrogram based on Ward's method indicated grouping of 22 genotypes of lentil into five clusters (Fig. 2). LM-123-7, LM-132-7, LM-13-1 and LM-24-3 were grouped in cluster I with high genetic (130) distance, while LM-28-2, LM-14-2, LM-21-6, LM-37-8, LM-48-1, LM-99-4, LM-95-3 and LM-75-4 in cluster II with 113 and LM-156-1, LM-185-2, LM-20-3 and LM-67-5 in cluster III with 113. ICARDA 2348 and ICARDA 23128 were grouped in cluster IV with highest genetic (219) distance. ICARDA 38211, ICARDA 23136, ICARDA 23121 and ICARDA 23105 were grouped in cluster V with lowest genetic (103) distance.

Gupta et al. 203

Dendrogram Ward's Method, Euclidean

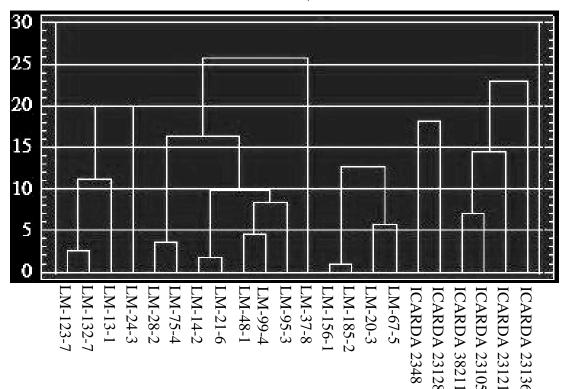


Fig. 2. Dendrogram based on summarized data on differentiation among 22 genotypes of lentil according to Ward's method

The mean values of each cluster for eleven characters are presented in Table 8. There was wide range of variation in the cluster mean values for all the characters. The mean values of all characters for the respective character were categorized into low (L), intermediate (I) and high (H) classes. Genotypes belong to cluster I showed high number of seed pod⁻¹, maximum 100-seed weight (g) and yield plant⁻¹ (g) then the rest of the clusters. Maximum days to maturity was found in cluster III then other cluster and minimum days to maturity was found in cluster I. Genotypes belong to cluster IV showed higher plant height (cm), days to flowering, number of secondary branches plant⁻¹, number of pod plant⁻¹ and number of seed plant⁻¹ then the other cluster. Genotypes belong to cluster V showed higher number of primary branches plant⁻¹ and height of the lowest pod.

Table 8. Cluster mean for 11 yield and yield related characters in 22 genotypes

Cluster	I	II	III	IV	V
Plant height (cm)	28.12 (I)	27.44 (L)	28.80 (I)	34.70 (H)	32.05 (I)
Days to flowering	51.95 (L)	56.00 (I)	59.20 (I)	68.70 (H)	64.65 (I)
Days to maturity	104.55 (L)	105.27 (I)	109.20 (H)	108.50 (I)	108.75 (I)
No. of primary branches plant ⁻¹	4.55 (I)	3.80 (L)	5.40 (I)	5.50 (I)	6.05 (H)
No. of secondary branches plant ⁻¹	11.80 (I)	9.53 (L)	12.00 (I)	15.70 (H)	14.90 (I)
Height of the lowest pod	6.17 (L)	6.70 (I)	6.53 (I)	8.95 (I)	11.55 (H)
Number of pod plant ⁻¹	181.55 (I)	136.40 (I)	160.30 (I)	264.70 (H)	117.45 (L)
Number of seed pod ⁻¹	1.58 (H)	1.51 (I)	1.41 (L)	1.51 (I)	1.45 (I)
Number of seed plant ⁻¹	292.60 (I)	205.68 (I)	233.25 (I)	401.30 (H)	168.05 (L)
100 seed weight (g)	2.55 (H)	2.21 (I)	2.01 (I)	1.77 (L)	2.02 (I)
Yield plant ⁻¹ (g)	7.46 (H)	4.65 (I)	4.78 (I)	6.89 (I)	3.40 (L)

H= High value, I= Intermediate value, and L= Low value

Table 9. Selection score, rank and expected genetic gain of 21 genotypes of lentil considering nine characters

SI. No.	Genotypes	Selection score	Rank	Expected genetic gain
1	LM-123-7	7636	1	
2	LM-132-7	6813	2	
3	LM-28-2	4716	7	
4	LM-14-2	5828	5	
5	LM-21-6	5952	3	
6	LM-24-3	5869	4	
7	LM-37-8	4759	6	
8	LM-48-1	4228	8	
9	LM-99-4	-167	10	110
10	LM-95-3	-231	14	143
11	LM-13-1	-252	15	
12	LM-156-1	-209	11	
13	LM-185-2	-286	16	
14	LM-20-3	-367	17	
15	LM-67-5	-223	13	
16	LM-75-4	-383	18	
17	ICARDA 2348	-215	12	
18	ICARDA 38211	401	9	
19	ICARDA 23121	-497	19	
20	ICARDA 23105	-577	21	
21	ICARDA 23128	-571	20	

Among 22 genotypes of lentil, LM-123-7 possessed the highest selection score index (7636) and rank as the best followed by LM-132-7, LM-21-6, LM-24-3 and LM-14-2 with 6813, 5952, 5869 and 5828 respectively (Table 9). The genotype ICARDA 23105 was worst having the lowest selection score of -577 followed by ICARDA 23128 (-571) and ICARDA 23121 (-497). The expected genetic gain (Δ G) was 143 at 5% selection intensity i.e. 2-3 highest scoring genotypes from these 21 lentil genotypes might be recommended for farmers' cultivation for better yield.

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