

GENETIC VARIATION IN LENTIL (*LENS CULINARIS* MEDIK) GERMPLASM EVALUATED UNDER IRRIGATED CONDITIONS OF AGRO-ECOLOGICAL UPLANDS OF QUETTA, BALOCHISTAN

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Abstract

Lentil is a main winter-sown legume crop widely grown in the Mediterranean type of climate, and has significant value as food, feed and fodder. Lentil often experiences considerable drought stress during growth season which negatively impact yield. Application of irrigation at crucial crop stages can stabilize productivity. The objective of this study was to elucidate the effect of irrigation on yield and study the correlated traits for indirect selection for yield. An experiment comprising eleven lentil genotypes including check variety Shiraz-96 was evaluated for yield and related traits under irrigation in randomized complete block designed repeated 3 times at Balochistan Agricultural Research and Development Center (BARDC), Quetta during 2018-19 season. The results indicate that significant genetic variation ($p \leq 0.05$) is present among lentil genotypes. Results revealed that all the traits exhibit significantly except days to flowering (50%). Maximum grain yield was recorded for genotypes 6 which produced 1030 kg ha⁻¹ grain yield and 3571 kg ha⁻¹ biological yield and 30% harvest index followed by genotype 2 with 1000 kg ha⁻¹ grain yield and 3183 kg ha⁻¹ biological yield with harvest index value of 31%. Grain yield also showed highly positive correlation with biological yield with a value of 0.84. Yield component trait of 100 seed weight depicted highest weight for genotype 5 with 3.52 grams followed by genotype 7 with 3.37 grams. Similarly, number of seeds per plant data showed maximum number of grains for genotype 10 producing 75 seeds per plant. Canopy temperature was also recorded to check the impact of heat on crop. The data on CT revealed genotype 6 as the coolest genotype which was also high yielding. Correlation data also showed moderate negative correlation of CT with grain yield -0.39. Days to flowering (50%) revealed non-significant difference with flowering range of 121 to 127 days. Cluster analysis showed four different groups within 2 major groups. Principal component analysis illustrated that first four components explained 83.9 percent variance with first two PCs explained the major variance of 31.5 and 26.6 variation.

Key words: *Camellia sinensis* L., Pharmacognostic, Phytochemical, Fluorescence Analysis, Powder Microscopy.

Introduction

Lentil (*Lens culinaris* Medik) is important food legume after beans, peas and chickpeas. It is mainly cultivated throughout the Mediterranean; Near East, East Africa, Middle East and the south Asia. It is traditionally grown as a rain fed crop, but in some areas, it is grown under supplemental irrigation. Globally, it is cultivated on 3.85 million hectares (m ha) area with 3.59 million tones (mt) production (Erskine *et al.*, 2009). Major geographical regions of lentil production are South Asia and China (44.3%), North America (41%), Central and West Asia and North Africa i.e. CWANA (6.7%), Sub-Saharan Africa (3.5%) and Australia (2.5%). Lentil is second most important pulse crop in winter in Pakistan after chickpea. Its seed is a rich source of protein (up to 28%), vitamins and minerals for human consumption, and the straw is a valuable animal feed. The crop is adapted to less favorable environments, where it is predominantly grown in winter under 200 to 400 mm rainfall (Sarker *et al.*, 2003). During selection process breeder aim to select germplasm with high and stable yield over seasons and environments. As yield is poly-genic trait it is highly influenced by environmental conditions which force breeders to pursue indirect selection where traits which are controlled by few genes but has high correlation with yield are selected.

Lentil is prone to drought stress in different growth stages with different degrees of negative influence on grain yield, mainly effecting grain yield at reproductive stage (Siddique *et al.*, 2000; Shrestha *et al.*, 2006a, b). Drought is one of the major abiotic stress which result in lower growth and fertility causing metabolic, physiological, morphological, and oxidative changes. Lentil is considered relatively resistant to drought but severe drought stress during specific developmental periods can cause significant yield and quality loss. The plants tolerate drought conditions by morphological changes such as stomatal closure, reduced leaf area and establishment of extensive root systems as result increase root/shoot ratio (Levitt, 1980). Wood (2007) explained the drought tolerance is the capability to maintain normal metabolism with low water potential during drought conditions. Drought stress decreases relative water content (RWC) of the leaves in plants which negatively impact rate of photosynthesis inhabiting photosynthetic activity. Plants adopt to drought by modifying specific cell and tissue physiology, molecular and biochemical changes. Reduce photosynthetic rate, regulation of anti-oxidative enzymes, accumulation of specific proteins, stress metabolites, stress regulatory gene expressions (Reddy *et al.*, 2004).

Geographically the Balochistan province falls in arid to semi-arid region. Soil condition, rainfall and desiccating winds are some of the factors influence lentil production in different regions of the Balochistan province, Pakistan which limits crop production. Lentil is one of the important winter pulse crops grown in parts of the province with great potential for increase. Agricultural statistics showed decreasing trend for lentil area due to increase in other crops like wheat. Lentil is cultivated on an area of 1677 hectares during 2018-19 which is 100 percent lower than 2008-09 with 3051 hectares planted producing 1928 tons during 2008-09 seasons (Agricultural Statistics Balochistan, 2018-19). Other issues for lentil area reduction include drought and unavailability of quality seed of high yielding varieties. Presently farmers are relying on low potential local varieties which are cold and drought tolerant but small seeded and produce lower yield in high rainfall years. Agrawal *et al.*, (2008) has reported that farmers can increase yield by 30-40% and even more if they combine the new varieties with improved crop management practices.

Present study was conducted to evaluate eleven lentil genotypes including long term check in agro-climatic condition of Quetta at PARC-Balochistan Agricultural Research and development center (PARC-BARDC) Quetta under irrigated condition to select high yielding germplasm and study traits having major influence on grain yield.

Material and Method

The trial was conducted to study the genetic variation in lentil germplasm grown under supplemental irrigation in agro-ecological upland of Balochistan (Quetta). The experiment was conducted at Pakistan Agricultural Research Council-Balochistan Agricultural Research and Development Centre (PARC-BARDC) Quetta during 2018-19. The trial was laid out in randomized complete block design (RCBD) comprising three replications with 11 genotypes (selected from previous year) including check variety Shir-Az-96 (Table 1). The plot length was 4 meters comprising four rows with row-to-row distance of 30 cm. All agronomic practices were kept same to reduce the environment influence. All four rows were harvested to record yield and related data. Data was recorded for traits like days to 50% flowering recorded from date of sowing when 50% flowers sprouted in each plot, plant height (cm) from base to top of the canopy, weight of 100 seeds (g), No of branches/plant, No of pods/plant, No. of seed/plant, canopy temperature (°C), biological yield (kg/ha), grain yield (kg/ha) and harvest index percentage respectively. Recorded data were statistically analyzed using analysis of variance method as proposed by Steel and Torrie (1986). Correlation analysis was also performed to evaluate traits with correlated effect with grain yield (Pearson's, 2008).

Steel, R.G. and Torrie, J.H., 1986. Principles and procedures of statistics: a biometrical approach. McGraw-Hill.

Table 1. List of genotypes and pedigree/parentage.

S. No.	Genotype	Pedigree and parentage
1.	BARDC-1	CIPAL203; Indian Head/ILL5588/ILL5588
2.	BARDC-2	ILL7685
3.	BARDC-3	ILL6467; ILL4605/ILL2582
4.	BARDC-4	ILL 46
5.	BARDC-5	ILL 262
6.	BARDC-6	ILL 7688
7.	BARDC-7	ILL 11
8.	BARDC-8	ILL 45
9.	BARDC-9	ILL 250
10.	BARDC-10	ILL8081
11.	Shir-Az-96	Check

Statistical analysis was carried out using “Statistica 8.1 and minitable 17”. Analysis of variance amongst characters was done by using SAS 9.0” and least significant difference (LSD $p \leq 0.05$) test was conducted for means evaluation to know the significant levels of treatment means (Jan *et al.*, 2009; Sharma *et al.*, 2016). The analysis also provided Principal components (Jolliffe, 1986) and cluster analysis to examine the influence of each PC and cluster genotypes according to similarity.

Result and Discussion

The analysis of variance revealed significant results ($p \leq 0.05$) for all tested traits excluding days to 50% flowering. Major traits like grain yield, biological yield, and number of grains per plant showed significant difference ($p \leq 0.05$) among genotypes (Fig. 1). Flowering data for different genotypes revealed a range of 121-127 days to flower (50%) which shows a narrow range of flowering. Plant height data showed maximum height for genotype BARDC-6 with 40 cm mean height followed by genotype BARDC-10 with plant height of 39.3 cm (Table 2). Genotype BARDC-6 also produced highest biological yield 3571 kg ha⁻¹ and grain yield 1030 kg ha⁻¹ (Fig. 1). The Pearson's correlation coefficient data showed (Table 4) moderate positive correlation for plant height with biological yield (0.33) and grain yield (0.19). The improvement in biomass has positive influence on final grain yield as it leads to further increase in N-assimilation and thereby in seed yield (Whitehead *et al.*, 2000). Mondal *et al.*, (2012) reported that better leaf area index may produce higher biological yield. Plant growth production and yield are characterized by the crop's capacity to intercept solar radiation which is further utilized for biomass production (Hanlan *et al.*, 2006). In lentil, better interception of solar radiation during early seedling stages facilitate plant to accelerate early growth, resulting in high yield (Purcell *et al.*, 2002). Harvest index (%) which is critical trait and define the partitioning of sink and source revealed maximum value for genotype BARDC-2 (31.3) followed by BARDC-4 (29.70) while minimum HI was observed for genotype BARDC-5 with 25.3% (Table 3). It is indicative that high partitioning efficiency (harvest index) would be useful for getting higher yield as shown for genotype BARDC-2 which stand second highest in grain yield (1000 kg ha⁻¹) display better partitioning of assimilates to sink. Harvest index (%) also depicted moderate positive correlation with grain yield (0.36) which

shows that selecting for higher HI is tantamount to select for higher grain yield (Table 4). Hundred seed weight which is an important yield related trait showed significant difference at $p \leq 0.05$. Highest hundred grain weight was recorded for genotype BARDC-5 (3.52) followed by genotype BARDC-7 (3.37). Our results depicted negative correlation of hundred grain weight with grain yield (-0.24). Due to high variability in BARDC soil and significant differences in micro and macro-sperma types of lentils the hundred grain weight was not correlated with yield. Grain numbers per plant is also important yield component trait and showed significant differences. Maximum number of seeds per plant were observed for genotype BARDC-10 (74) followed by BARDC-2 (67) grains per plant. Number of seed per plant showed high positive correlation with days to 50% flowering showing that late flowering genotypes produced larger number of flowers hence resulting in higher number of grains. Number of seeds per plant revealed low but negative correlation with hundred grain weight. Due to high variability in macro and microsperma types both grain weight and grain numbers per plant showed smaller negative correlation. Further evaluation of these traits for second year can provide better insight for understanding influence of environment and genetic architecture. Longer crop period in Quetta due to its environmental conditions and steady increase in temperature, plant tend to develop maximum number of flowers which in turn produce

maximum number of seed without aborting. Similar results were shown by Tambal *et al.*, (2000). The correlation coefficient for grain yield with number of pods per plant and seeds per pod was negative (Table 4) which may be the reason of difference in seed size as reported by Bacchi *et al.*, (2010) who indicate that microsperma has higher influence on yield as compared to macrosperma. Number of branches plant⁻¹ was also evaluated. The data showed significant difference among lentil genotypes for this trait with maximum number of 7 branches recorded for check Shir-Az-96 and genotype BARDC-6 each. Correlation data revealed positive correlation of NBP⁻¹ (numbers of branches per plant) with biological yield (0.12), grain yield (0.26) and HI (0.33). Furthermore, canopy temperature was also taken to study the impact of stress during crop growth. CT is considered as proxy trait to root growth/stomatal conductance as canopy with cooler temperature is tend to be efficient in transpiration while hotter canopies show the incidence of stress. Our results depicted significant ($p \leq 0.05$) difference among genotypes for CT with a range of 22 to 29°C. CT showed negative correlation with grain yield (-0.39) which indicate that cooler canopies result in better stomatal conductance hence improving final grain yield (Table 4). Blum *et al.*, (1989) reported positive correlation ($r=0.65$) of drought susceptibility index and canopy temperature in a study carried out in wheat revealing that genotypes with warmer canopies tend to yield low as compared to genotypes with cooler canopies.

Table 2. Average data of multiple morphological and yield related traits for different lentil genotypes during the winter season 2018-19 at BARDC Quetta.

S. No	Genotype	Days to flowering (50%)	Plant height (cm)	Hundred seed weight (g)	Number of branches plant ⁻¹	Number of pods plant ⁻¹	Number of seed plant ⁻¹	Canopy temp. (°C)
1.	BARDC-1	122.00	28.66 c	2.98ab	5.67abc	35.0abc	58.67 b	26.40ab
2.	BARDC-2	123.67	28.33 c	2.99ab	6.67ab	36.3abc	67.33ab	26.50ab
3.	BARDC-3	122.33	28.33 c	2.80 b	5.00 c	36.3abc	65.67ab	26.90ab
4.	BARDC-4	121.67	28.33 c	3.27ab	5.00 c	35.6abc	64.33ab	27.40ab
5.	BARDC-5	123.33	34.66abc	3.52a	6.00abc	37.3abc	65.17ab	29.03a
6.	BARDC-6	124.33	40.00a	2.77 b	6.67ab	35.6abc	62.33ab	22.50 b
7.	BARDC-7	125.00	36.00ab	3.37ab	5.33 bc	38.0abc	64.17ab	26.80ab
8.	BARDC-8	122.33	29.33 c	2.82ab	5.33 bc	34.0 bc	60.17ab	24.17ab
9.	BARDC-9	125.33	34.33abc	3.35ab	5.67abc	32.0 c	62.33ab	24.67ab
10.	BARDC-10	127.00	39.33a	2.82ab	5.67abc	42.3 a	74.67a	24.73ab
11.	ShirAz-96	121.67	35.00abc	3.24ab	7.00a	40.0 ab	66.67ab	25.50ab
LSD value: (0.05%)		N.S	7.32	0.7135	1.53	7.88	15.56	5.42

Values within the same column followed with the same letters are not significantly different using LSD range test at 5% level

Table 3. Average data of yield and its components for different lentil genotypes during the winter season 2018-19 at BARDC Quetta.

S. No.	Genotype	Biological yield (kg ha ⁻¹)	Grain yield (Kg ha ⁻¹)	Harvest index (%)
1.	BARDC-1	2976.2 abc	882.4 ab	27.22 ab
2.	BARDC-2	3183.0 abc	1000.0ab	31.31 a
3.	BARDC-3	2996.3 abc	883.1 ab	27.86 ab
4.	BARDC-4	2797.7 bc	773.7 b	29.70 ab
5.	BARDC-5	3161.5 abc	837.7 ab	25.34 b
6.	BARDC-6	3571.4 a	1030.7 a	29.54 ab
7.	BARDC-7	3091.3 abc	928.0 ab	28.37 ab
8.	BARDC-8	3170.1 abc	839.6 ab	26.50 ab
9.	BARDC-9	3452.0 ab	964.8 ab	27.62 ab
10.	BARDC-10	2989.1 bc	829.7 ab	28.24 ab
11.	ShirAz-96	2639.8 c	759.7 b	28.55 ab
LSD value: (0.05%)		724.59	241.79	5.52

Values within the same column followed with the same letters are not significantly different using LSD range test at 5% level

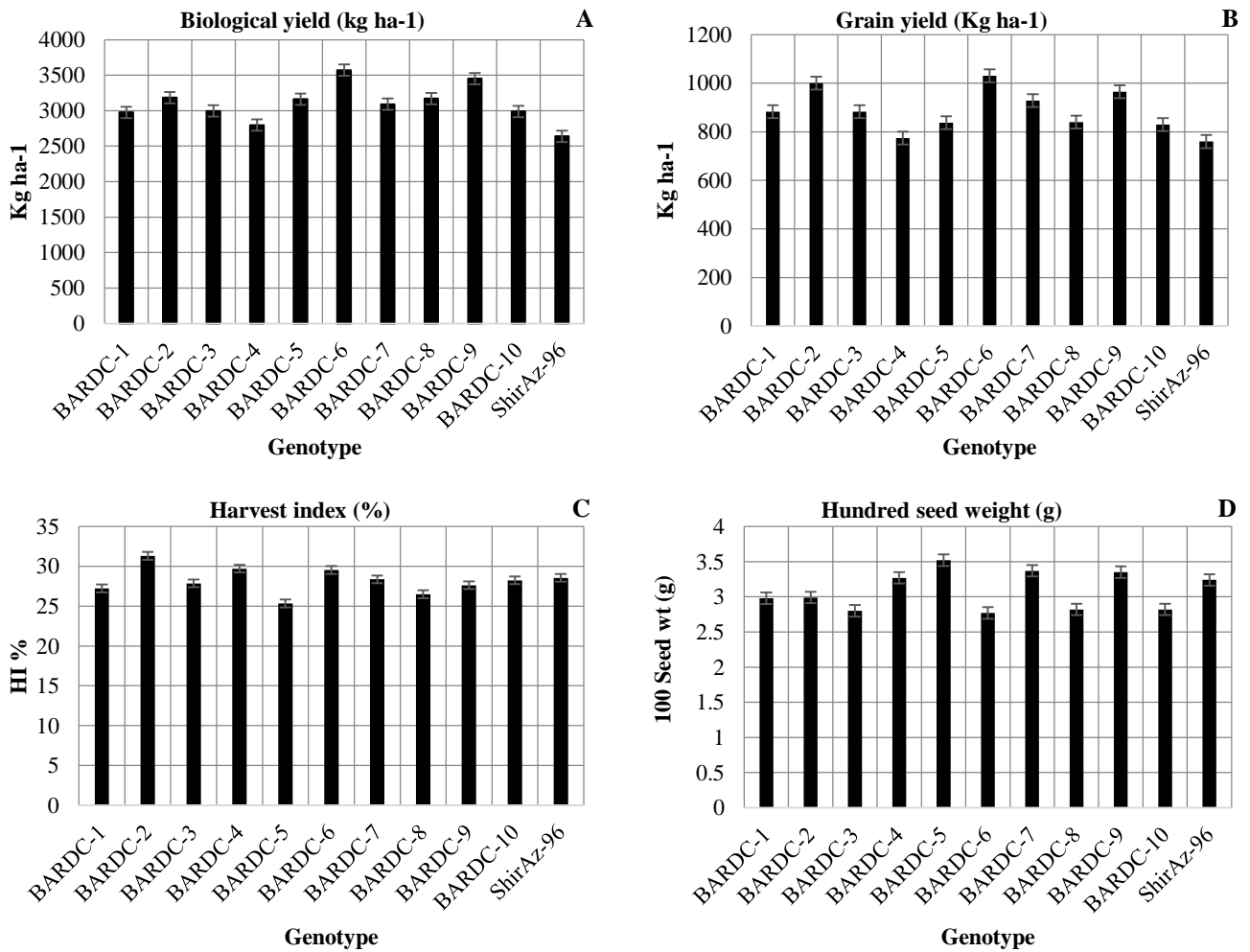


Fig. 1. a, b, c and d Mean bar graphs for Biological yield kg ha⁻¹, Grain yield kg ha⁻¹, Harvest index (%) and Hundred Seed weight (g).

Table 4. Phenotypic Pearson’s correlation among yield and yield related traits.

Trait	PH (cm)	100 SWt (g)	NBP ⁻¹	NPP ⁻¹	NSP ⁻¹	CT (°C)	BY (Kg ha ⁻¹)	GY (Kg ha ⁻¹)	HI (%)
100 SWt (g)	0.06								
NBP ⁻¹	0.39	0.00							
NPP ⁻¹	0.47	-0.05	0.25						
NSP ⁻¹	0.39	-0.08	0.15	0.82					
CT (°C)	-0.45	0.61	-0.30	0.12	0.08				
BY (Kg ha ⁻¹)	0.33	-0.16	0.12	-0.53	-0.29	-0.47			
GY (Kg ha ⁻¹)	0.19	-0.24	0.26	-0.41	-0.22	-0.39	0.84		
HI (%)	-0.06	-0.25	0.33	0.11	0.27	-0.23	-0.02	0.36	
DTF (50%)	0.70	-0.04	0.03	0.29	0.52	-0.33	0.49	0.43	0.07

PH.Plant Height; SWt. 100 Seed Weight; NBP. Number of Branches per plant; NPP. Number of pods per plant; NSP. Number of Seeds per Plant; CT. Canopy Temperature; BY. Biological Yield; GY. Grain Yield; HI. Harvest Index (%)

Principal component analysis: Principal component analysis showed that at BARDC, Quetta, the first four PCs explained most of the total variance (Table 5), which was 83.9% with individual values (31.5, 26.6, 14.8 and 11 for 4 PCs respectively). The major traits related to yield like biological yield (0.424) and grain yield (0.428) explained positively by contributing to first principal component. Plant height also contributed positively to first PC. In a study carried out at Spain, the morpho-agronomical characters different lentil landraces revealed

that the first five components of PCA explained 83.7% of the cumulative variance and seed production contributed the most to the first factor (Cristobal *et al.*, 2014). Likewise, yield and yield components accounted for 73.13% of the total variance in the first three components of PCA (Toklu *et al.*, 2009).

Loading plot: Loading plot graphs the coefficients of each variable for the first component versus the coefficients for the second component. Loading plot provide information to

identify which variables have largest effect on each component. Loadings can range from -1 to 1. Loadings closer to -1 or 1 shows the variable strongly influence the principal component. The angles of loading provide how closely correlated variables are. The results from loading plot (Fig. 2) revealed that grain yield and biological yield clustered at similar region showing higher correlation and had major influence on first component. Similarly, Number of pods per plant and number of seeds per plant were clustered together. The figure also showed canopy temperature having negative correlation with biological yield and grain yield which is also calculated using correlation (Table 4). Pradhan *et al.*, (2020) reported that canopy temperature has negative association with yield as cooler canopies tend to yield more with efficient stomatal conductance mainly under stress conditions. Biological yield has positive influence on final

grain yield which indicate that better radiation capture and transfer of assimilates plays major role in crop development and final grain yield (Whitehead *et al.*, 2000 and Hanlan *et al.*, 2006).

Cluster analysis: The cluster analysis depicted two major cluster with second cluster subdivided in further 3 clusters (Fig. 3). Cluster one comprises of genotype BARDC-6 and BARDC-9 with level of similarity of 85.92 while second cluster comprised of 1, 3 and 10 in first sub-cluster, 2, 7, 5 and 8 in second sub-cluster while 4 and 11 in 3rd sub-cluster. Level of similarity for genotype BARDC-5 and BARDC-8 was maximum with 98.6 score followed by genotype 1 and 3 with 97.7 score (Table 6). Genotype BARDC-1 and BARDC-6 were least similar and similarity score was 0.

Table 5. Eigenvalue, percent of variance, cumulative variance and loading of the traits of the first four principal components (PCs) of tested lentil genotypes grown at BARDC, Quetta.

Parameters	PC1	PC2	PC3	PC4
Eigenvalue	3.15	2.66	1.48	1.09
Percent variance explained	31.5	26.6	14.8	11.0
Cumulative variance explained	31.5	58.1	73.0	84.0
Variable	Eigen vector			
Days to flowering (50%)	0.406	0.216	0.357	0.163
Plant height (cm)	0.375	0.295	0.331	-0.021
Hundred seed weight (g)	-0.220	0.051	0.520	-0.577
Number of branches plant	0.244	0.141	-0.220	-0.621
Number of pods plant	0.007	0.590	-0.065	0.053
Number of seed plant	0.101	0.546	-0.048	0.079
Canopy temperature	-0.420	0.089	0.231	-0.292
Biological yield (Kg ha ⁻¹)	0.424	-0.323	0.259	-0.018
Grain Yield (Kg ha ⁻¹)	0.428	-0.282	-0.001	-0.214
Harvest index (%)	0.192	0.076	-0.564	-0.339

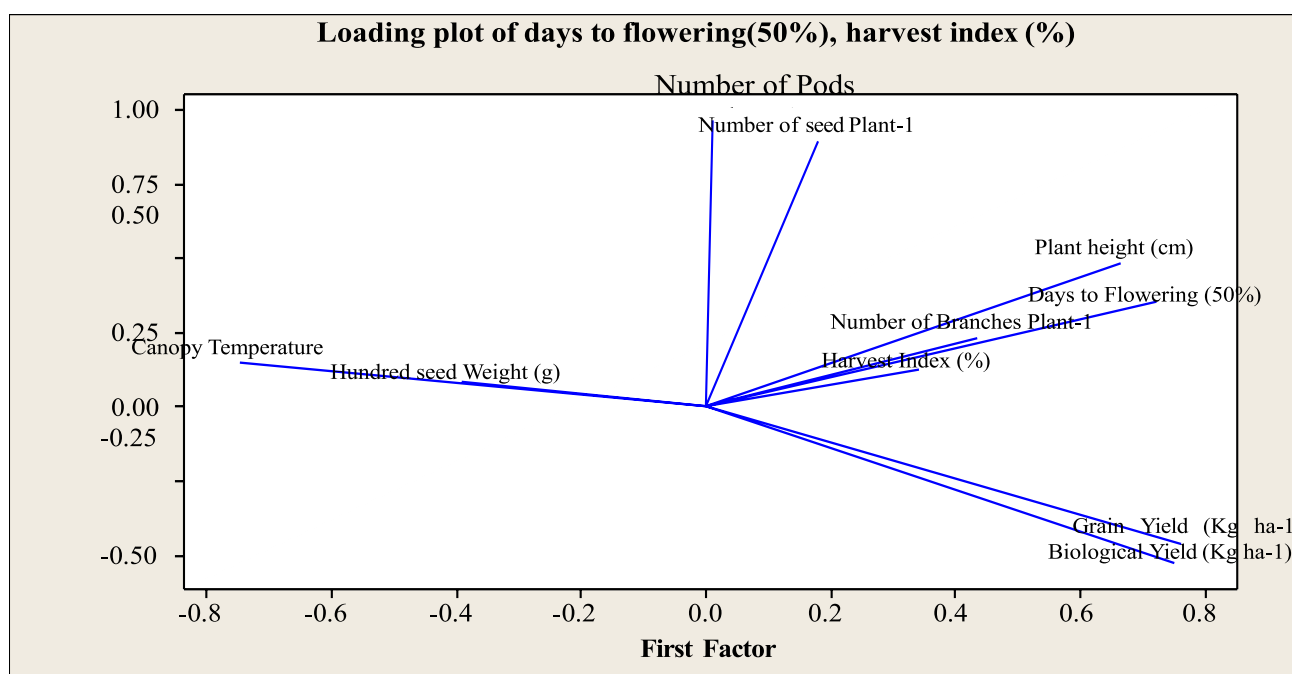


Fig. 2. Scattered diagram of Lintel (*Lens culinaris* Medik) genotypes for agronomic traits for first two PCs.

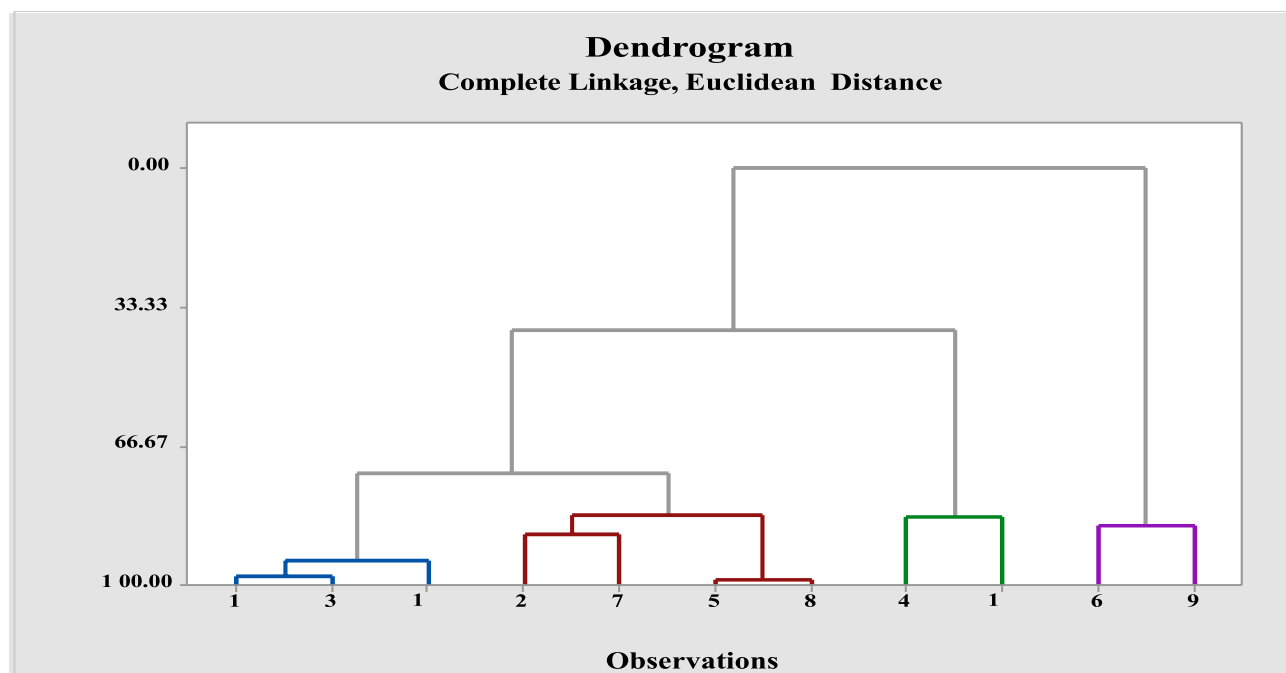


Fig. 3. Dendrogram derived from analysis of eleven lentil (*Lens culinaris* Medik) genotypes.

Table 6. Average level of similarity, level of Euclidean distance and complete linkage amalgamation steps of lentil (*Lens culinaris* Medik) genotypes.

Step	Level of similarity	Level of euclidean distance	Clusters joined (genotypes)	
1	98.6603	12.999	5	8
2	97.7976	21.369	1	3
3	93.9937	58.276	1	10
4	87.9467	116.948	2	7
5	85.9228	136.585	6	9
6	83.6369	158.764	4	11
7	83.0985	163.988	2	5
8	73.3551	258.524	1	2
9	38.7752	594.038	1	4
10	0.0000	970.257	1	6

Conclusion

In this study we observed significant variability in lentil genotypes evaluated under irrigated condition. Grain yield is a poly-genic trait and require rigorous selection and evaluation to select genetically stable and better performing genotype. To reduce the selection time and fastened development of high yielding genotypes indirect selection traits can play critical role in improving breeding program. In this study multiple analytical tools were used to observe the association of different morphological traits with yield. Our results revealed that genotype BARDC-6 and BARDC-2 has the potential to be advanced for next growing cycle and may produce stable yield which can make it candidate for development as new variety. Furthermore, traits like harvest index and canopy temperature can be used in indirect selection as they showed higher association with grain yield. Further studies are required to check the influence of environment and genotype and environment interaction and separate the actual genetic effect with selection of stable genotype for development of new lentil variety.

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