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Genetic Variability of Physiochemical Traits in Exotic and Indigenous Lentil (*Lens culinaris* L.) Genotypes

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ABSTRACT

Assessment of different physical attributes and hydration properties of lentil is imperative for its storage, cooking quality and market acceptance. Present study evaluated the genetic diversity based on different seed hydration properties among 59 lentil genotypes including exotic (43) and indigenous lines/cultivars (16). The 100- seed weight (HSW), hydration capacity (HC), hydration index (HI), seed volume (SV), swelling capacity (SC), and swelling index (SI) were examined in a complete randomized design (CRD). Significant genotypic differences were observed for the studied traits. Highest HSW (4.9 g), maximum SV (0.041 ml seed^{-1}) and HC (0.0734 ml seed^{-1}) were exhibited by exotic genotype (ILL4402×ILL5588), after 24 hours of soaking. Besides, MANSEHRA 89 and NIA-Masoor 2016 outperformed other genotype for SC (0.0715 ml seed^{-1}) and SI (0.0337), respectively. Maximum value for HI (0.0213) and SI (0.025) were presented by exotic lines (ILL10731×ILL4637 and ILL4605×ILL7950), respectively. The correlation analysis revealed significant positive correlation between HSW with HC (0.811) SV (0.524) and SC (0.196), while SV showed significant positive correlation with HC (0.404). In addition, SC and HC also showed significant positive association (0.203). Heritability analysis revealed that, HSW exhibited highest heritability (30.81) and genetic advance (34.37), followed by HI and SI. The Principle component analysis further concluded the first three components (PC1, PC2 and PC3) explained about 91.94% of the variation. It is concluded that the identification of superior exotic and existing cultivars through hybridization can broaden the genetic base. Further, the genotypes with desired hydration properties identified in this study can serve as promising source of variability for efficient utilization in future lentil breeding and crop improvement.

Keywords: Genetic diversity, Heritability, Hydration properties, Cooking quality, Swelling Index.

INTRODUCTION

Lentil (*Lens culinaris* L. Medikus) is a winter season, self-pollinating annual crop and ranked 5th in global production among leguminous crops. It plays an important part as human diet and animal feed and it provides important dietary source of energy, iron, carbohydrates, minerals, plant-based proteins, vitamins, potassium, fiber and antioxidant compounds, in addition to diverse non-nutritional constituents (Pandey and Sengar, 2020; Laskar et al., 2019). As it contains lesser amounts of fats, it also plays significant role in controlling the risk of some diseases as coronary, obesity, risk of type 2 diabetes and heart disorders (Chelladurai and Erkinbaev, 2020).

Lentil (*Lens culinaris* Medikus) annual production is approximately 4.5 million tonnes (Chelladurai and Erkinbaev, 2020). Worldwide production is observed twice in the last two decades, from 3.15 million metric tons (MMT) to 6.54 MMT from 2001 to 2020, respectively (FAO, 2021). Despite the fact that improved genotypes account for the majority of the contribution to lentil yield and output, yet in most lentil production sites, the actual yield lags behind the potential yield by approximately 50% (Ghaffar et al., 2023). Although many efforts have been made regards to lentil production with maximum rate, still more work needs to be done for the progress in quality production by introducing some genetic variations.

Seed quality and cooking quality are considered imperative for the market acceptance. Moreover, the choice of consumers also serves as a determining factor based on the cooking characteristics and quality parameters. Despite the proven nutritional significance of pulses, the higher soaking time and cooking time undermines their utilization. The rate of water uptake and hydration properties affects the seed hardness, which in turn affects the legumes cooking time (Chen et al., 1993). Generally, the rate of water uptake corresponds to less cooking time. Genetic analysis plays an important role for the selection of the desired genotypes comprising of various quality traits. These variations depend on numerous factors such as seed size, color of the seed, seed pattern, hydration properties, seed weight, swelling capacity and genetic characteristics along with some environmental factors. For an instance, Singh et al. (2004) reported that reduced hydration properties are linked with sub-optimal cooking quality and low milling efficiency further affecting in the end-use properties in black gram. Similarly Joshi et al. (2010) also investigated the water uptake as a measure of hydration capacity, in relation with temperature and soaking time in lentil genotypes. Thus, it is imperative to screen the lentil germplasm for desired physiochemical attributes, for meeting the consumer's demand and economic gains.

The negative impacts of diverse abiotic pressures on agricultural output can be reduced with a combination of genetic innovation and cultural approaches (Aktar et al., 2022). Several studies have been carried out for estimation of the variability in physiochemical traits in chickpea and (Malik et al., 2011; Sastry et al., 2019). Oskaybaş et al. (2021) studied the physiochemical and nutrition importance of lentil and its use in quality cooking. Taleb et al. (2016) also demonstrated the evaluation of three genotypes of lentil for their physiochemical attributes such as 100-seed weight, seed volume, water absorption capacity, bulk density, turgidity index, water absorption index, grain turgidity and true density. It was further corroborated that the HSW can be a significant indicator in analyzing and representing the density of seeds, seed size and quality which are being affected by some of external as well as genetic factors (ICARDA 2004). Bicer and Sakar, (2010) have also reported the significant genotypic variation for 1000-seed weight among 10 different genotypes of lentils in Turkey with heritability of 98% for the seed weight. Similarly, a considerable difference between the two genotypes in terms of some physical characteristics, including 1000-seed weight, bulk density, and porosity was also reported by Szot et al. (2003). In addition, several studies have reported the genotypic and phenotypic differences for the seed weight and physiochemical attributes in lentil in terms of higher heritability to improve these in lentil (Adewale et al., 2010; Singh et al., 2012). The purpose of the present study was to analyze the genetic variance of the physiochemical traits in indigenous and exotic lentil germplasm and cultivars for its effective utilization in improving the cooking quality and hydration properties of lentils.

MATERIALS AND METHODS

Experimental details

A total 59 lentil accession, 16 locally released varieties of lentil (including one advanced line from NARC), 42 accessions acquired from ICARDA and one (1) cultivar from Australia, were evaluated for the genetic diversity of different hydration related traits. This study was performed in a complete randomized design (CRD) during 2023 at Pulses breeding lab, NARC, Islamabad under natural room conditions. The seed traits taken for the study of seed quality were 100-seed weight (HSW), seed volume (SV), hydration index (HI), hydration capacity (HC), swelling capacity (SC) and swelling index (SI) by using the protocol designated by (Williams et al., 1983; Malik et al., 2011).

Estimation of seed characteristics

A total of 100 seeds from each genotype were selected randomly for the estimation of 100-seed weight (HSW). Additionally, seed volume (SV) was estimated by placing 100 seeds into a measuring cylinder comprising of desired volume of distilled water (25 ml) by using the following formula:

$$Seed\ Volume = \frac{(\text{Total volume} - 25)}{100}$$

Seed hydration capacity (HC) was estimated by percentage using the following formula:

$$HC = \frac{(Wf - W0)}{100}$$

Where, W_f represents the 100 seed weight after soaking for 24 h While W_0 is the 100 seed weight before soaking. Seed hydration index (HI) was calculated as the proportion in hydration capacity and seed weight and assessed by following the equation given below:

$$HI = \frac{Hydration\ capacity}{Seed\ Weight}$$

Swelling capacity (SC) of the seeds was computed by the formula:

$$SC = \frac{(Vf - V0)}{100}$$

Where, V_f is the 100 seed volume of soaked seeds for 24 h While, V_0 is the 100 seed volume before seeds were soaked. Seed swelling index (SI) was observed as ratio between swelling capacity and seed volume and assessed by the equation given below:

$$SI = \frac{Swelling\ capacity}{Seed\ Weight}$$

Statistical analysis

Analysis of variance was performed for the determination of significant differences between the studied genotypes (Steel and Torrie, 1997). Principle component analysis (PCA) and correlation coefficients were computed by using R software and R Studio (RStudio Team, 2020).

RESULTS

Determination of analysis of variance and mean performance

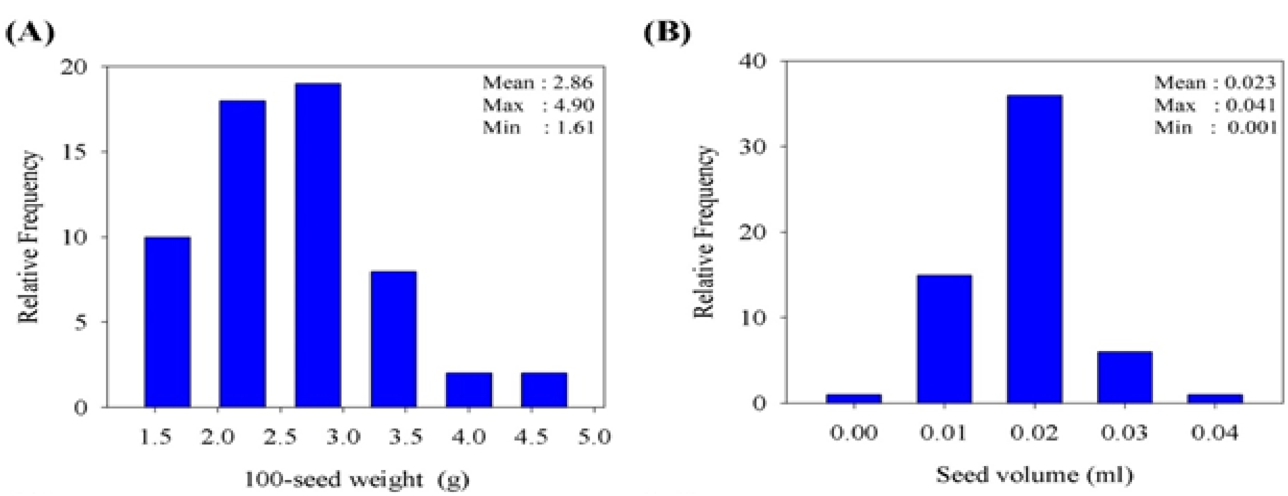
A total of 59 exotic and indigenous accessions of lentil were examined for various physiochemical attributes. Frequency distribution for each class of the studies attributes are compiled in Table 1. Significant genotypic variances were observed among all the 59 lentil genotypes. For instance, average HSW ranged from 1.6g (NIA Masoor 06) to 4.9g (ILL4402×ILL5588), indicating the larger amount of variability present among genotypes (Table 1). On contrary, the average HSW of Hurricane (Australian cultivar) was 2.98 g. Maximum seed volume was recorded in ILL4402×ILL5588 (0.041 ml seed^{-1}) followed by ILL10750×ILL1959 (0.0385 ml seed^{-1}) while the minimum SV was shown by ILL5588 (0.001 ml seed^{-1}) (Table 1). On the other hand, HC of ILL4402×ILL5588 (0.0734 g seed^{-1}) ranked highest, followed by ILL10750×ILL1959 (0.0692 g seed^{-1}) while minimum HC was recorded in NIAB MASOOR 06 (0.0285 g seed^{-1}). Similarly, ILL10731×ILL4637 and NIA Masoor 2016 showed maximum value for HI, respectively. Besides, lowest HI was exhibited by FLIP97-34L×FLIP97-33L (0.0117). In contrast, maximum value of SC was observed in MANSEHRA 89 (0.0715 ml seed^{-1}) followed by FLIP97-34L×FLIP97-33L (0.0685 ml seed^{-1}) while Sheraz showed least SC value (0.029 ml seed^{-1}). ILL4605×ILL7950 displayed highest SI (0.0250) followed by ILL8620 (0.0244) (Table 1). Similarly, the relative frequency of the lentil accessions for different studied traits also indicated the wide range of variability present in the studied germplasm (Figure 1).

Table 1: Mean values of parameters for different genotypes. 100-seed weight (HSW), Hydration Capacity (HC), Hydration Index (HI), Swelling capacity (SC), Seed volume (SV), Swelling Index (SI).

Sr. #	Accession(s)	Origin /Source	HSW (g)	SV (ml)	HC (g seed^{-1})	HI	SC (ml seed^{-1})	SI
1.	FLIP96-49L×FLIP97-33L	ICARDA	3.37	0.023	0.0464	0.0138	0.035	0.0104
2.	FLIP97-29L×FLIP97-33L	ICARDA	3.22	0.021	0.0436	0.0136	0.044	0.0137
3.	ILL4402×ILL7979	ICARDA	3.31	0.029	0.0494	0.0149	0.044	0.0133
4.	ILL10749×ILL3597	ICARDA	2.91	0.021	0.0468	0.0161	0.047	0.0162
5.	ILL10801×ILL2711	ICARDA	3.54	0.030	0.0534	0.0151	0.039	0.0110
6.	ILL10750×ILL1959	ICARDA	4.80	0.039	0.0692	0.0144	0.054	0.0113
7.	ILL4605×BARIMUSOR-6	ICARDA	3.58	0.025	0.0537	0.0150	0.053	0.0148
8.	BARIMUSOR- 6×L-7713	ICARDA	3.35	0.028	0.0531	0.0159	0.043	0.0128
9.	L-4147×ILL4649	ICARDA	3.24	0.030	0.0456	0.0141	0.046	0.0140
10	L-4147×ILL4649	ICARDA	4.25	0.038	0.0592	0.0139	0.053	0.0124
11	ILL10800×ILL4419	ICARDA	3.07	0.030	0.0482	0.0157	0.050	0.0163
12	FLIP97-34L×FLIP97-33L	ICARDA	3.42	0.028	0.0546	0.0161	0.037	0.0108
13	BARIMUSOR-6 ×LIRL-21-50-1-1-1-0	ICARDA	2.76	0.020	0.0518	0.0194	0.048	0.0173
14	ILL7177	ICARDA	2.60	0.019	0.0421	0.0164	0.053	0.0204
15	ILL7947	ICARDA	2.77	0.023	0.0423	0.0153	0.036	0.0130
16	ILL11135	ICARDA	2.88	0.019	0.0472	0.0164	0.047	0.0162
17	ILL8620	ICARDA	2.01	0.020	0.0348	0.0173	0.049	0.0244
18	ILL5582	ICARDA	3.07	0.019	0.0474	0.0156	0.044	0.0145
19	ILL5883	ICARDA	3.04	0.023	0.0472	0.0155	0.043	0.0140
20	ILL7010	ICARDA	3.00	0.020	0.0471	0.0157	0.050	0.0167
21	ILL6246	ICARDA	2.63	0.014	0.0447	0.0169	0.040	0.0152
22	ILL7513	ICARDA	3.38	0.023	0.0503	0.0149	0.063	0.0185
23	ILL11029	ICARDA	2.37	0.010	0.0420	0.0177	0.043	0.0182

24	ILL5588	ICARDA	2.19	0.001	0.0389	0.0177	0.048	0.0216
25	ILL10800×33109	ICARDA	2.33	0.015	0.0393	0.0168	0.038	0.0162
26	ILL4402×ILL5588	ICARDA	4.90	0.041	0.0734	0.0149	0.062	0.0126
27	FLIP96-49L×FLIP97-33L	ICARDA	3.52	0.025	0.0438	0.0125	0.033	0.0092
28	FLIP96-49L×FLIP97-34L	ICARDA	2.91	0.023	0.0484	0.0166	0.063	0.0217
29	ILL7723×ILL8010	ICARDA	2.78	0.025	0.0518	0.0188	0.066	0.0238
30	KHAJURA-2×ILL5888	ICARDA	2.65	0.024	0.0398	0.0150	0.047	0.0176
31	ILL7723×ILL5244	ICARDA	2.83	0.019	0.0459	0.0162	0.047	0.0166
32	BARIMUSOR- 6×ILL6994	ICARDA	2.44	0.015	0.0447	0.0183	0.053	0.0215
33	ILL7978×ILL7537	ICARDA	3.57	0.030	0.0543	0.0152	0.036	0.0100
34	ILL10731×ILL4637	ICARDA	2.22	0.013	0.0468	0.0213	0.035	0.0159
35	FLIP97-34L×FLIP97-33L	ICARDA	3.89	0.032	0.0454	0.0117	0.069	0.0176
36	ILL8007×ILL759	ICARDA	2.96	0.022	0.0418	0.0141	0.034	0.0114
37	FLIP97-29L×FLIP97-33L	ICARDA	3.42	0.025	0.0488	0.0143	0.063	0.0183
38	ILL5883×ILL10750	ICARDA	3.17	0.025	0.0506	0.0159	0.058	0.0183
39	ILL10800×ILL4637	ICARDA	3.09	0.026	0.0435	0.0140	0.056	0.0179
40	ILL4605×ILL6024	ICARDA	4.22	0.025	0.0561	0.0133	0.040	0.0095
41	BARIMUSOR- 6×ILL6994	ICARDA	3.52	0.025	0.0532	0.0151	0.050	0.0142
42	ILL4402×ILL5588	ICARDA	2.92	0.020	0.0439	0.0150	0.055	0.0188
43	ILL4605×ILL7950	ICARDA	2.21	0.021	0.0324	0.0147	0.055	0.0250
44	Markaz 09	Pakistan	2.33	0.031	0.0348	0.0149	0.046	0.0196
45	NIA Masoor 06	Pakistan	1.62	0.025	0.0307	0.0189	0.051	0.0313
46	MANSEHRA 89	Pakistan	2.52	0.026	0.0442	0.0174	0.072	0.0289
47	Punjab Masoor 2020	Pakistan	2.43	0.020	0.0371	0.0153	0.037	0.0150
48	Punjab Masoor 2019	Pakistan	2.09	0.015	0.0377	0.0179	0.041	0.0195
49	Punjab Masoor 2009	Pakistan	2.29	0.029	0.0372	0.0162	0.046	0.0200
50	NIAB MASOOR 06	Pakistan	1.84	0.015	0.0285	0.0155	0.038	0.0204
51	NIAB MASOOR 2002	Pakistan	1.95	0.015	0.0307	0.0158	0.040	0.0206
52	Masoor 85	Pakistan	1.91	0.015	0.0363	0.0190	0.038	0.0200
53	Masoor 04	Pakistan	1.78	0.020	0.0321	0.0181	0.033	0.0185
54	LS-18-02 (NARC advanced line)	Pakistan	2.53	0.020	0.0375	0.0148	0.053	0.0207
55	Chakwal Masoor	Pakistan	2.50	0.023	0.0361	0.0144	0.039	0.0154
56	Masoor 93	Pakistan	1.70	0.015	0.0311	0.0183	0.049	0.0285
57	Sheraz	Pakistan	2.20	0.028	0.0366	0.0166	0.029	0.0132
58	Hurricane	Australia	2.98	0.030	0.0479	0.0161	0.038	0.0128
59	NIA Masoor 2016	Pakistan	1.86	0.028	0.0361	0.0194	0.063	0.0337
	Mean Squares		1.561***	0.0001***	0.0002***	0.0004***	0.0003***	0.0001***
	CV %		4.4	22.8	10.5	11.4	8.6	23.0

***: Level of significant differences at $p<0.001$



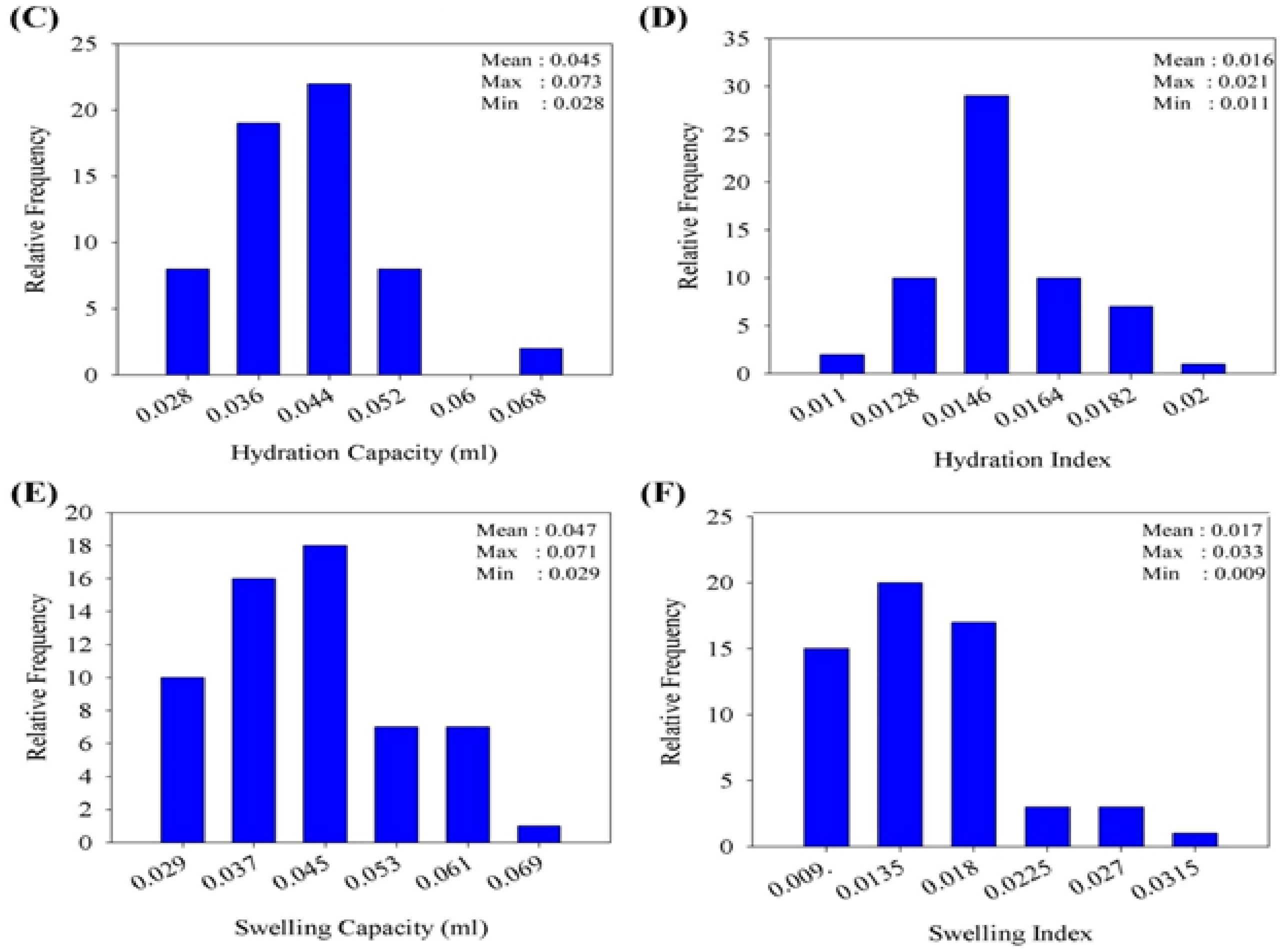


Figure 1. Relative frequency of lentil accessions for different Physiochemical traits.
(A) 100-seed weight, (B) Seed volume, (C) Hydration capacity, (D) Hydration index, (E) Swelling capacity and (F) Swelling index, with their range and mean values.

Table 2: Genetic analysis of seed physical quality traits. Standard Error (S.E), Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV), Heritability (H^2), Genetic Advance (GA).

Character(s)	Mean	S.E	GCV%	PCV%	H^2 (B.S)	GA
100 Seed Weight (g)	2.86	0.36	10.80	21.34	30.81	34.37
Seed volume (ml $seed^{-1}$)	0.023	0.007	17.61	5.06	0.17	0.002
Hydration Capacity (g $seed^{-1}$)	0.045	0.007	12.25	3.43	0.22	0.003
Hydration Index	0.016	0.002	20.51	5.75	0.62	0.026
Swelling Capacity (ml $seed^{-1}$)	0.047	0.008	12.79	3.83	0.21	0.004
Swelling Index	0.017	0.003	13.34	2.53	0.26	0.002

Determination of correlation for different seed hydration properties

While estimating correlation for the studied traits, it was observed that HSW exhibited significant negative correlation with SI (-0.653) and HI (-0.506) while significant positive correlation with HC (0.811) and SC (0.196) was shown (Figure 2). Hydration capacity exhibited highly negative correlation with swelling index (-0.492) while showed significant positive correlation with seed volume (0.404). In case of hydration index and hydration capacity, non-significant correlation was concluded. Hydration index showed significant positive association with swelling index (0.432) and negative significant negative association with seed volume (-0.299) while HI and swelling capacity (-0.010) were negatively correlated (Figure 2). Swelling capacity exhibited high positive correlation with swelling index (0.564) and non-significant association with seed volume (0.041). Seed volume showed highly negative correlation (-0.347) with swelling index (Figure 2).

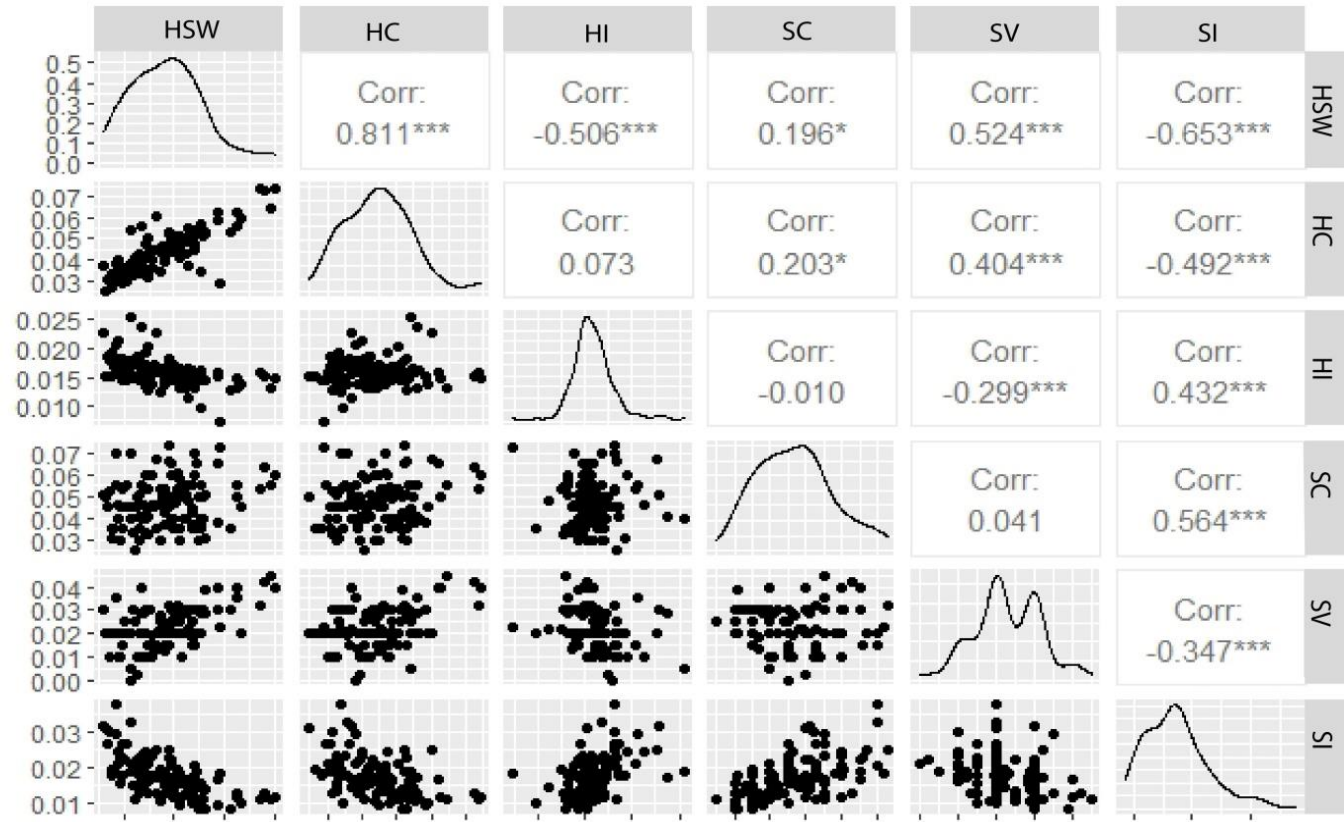


Figure 2. Correlation coefficients among different physiochemical traits in lentil accessions.
HSW=100-seed weight, SV=Seed volume, HC=Hydration capacity, HI=Hydration index, SC=welling capacity and SI=Swelling index

Principle component analysis and clustering

Principal Component Analysis (PCA), a multivariate statistical method, was employed to examine the association among traits and between traits and observations. The PCA was conducted using Pearson's correlation coefficient matrix. Communalities were computed to assess the proportion of variance explained by Principal Components (PCs). The first two components (PC1 and PC2) explain about 79.96% of the total variability, while the first three (PC1, PC2, and PC3) explain about 91.94% (Table 3).

Table 3. Estimated major principal components and their communalities for respective variables and principle components (PCs).

Parameters	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	1.8147	1.2266	0.8477	0.67056	0.17134	0.06992
Variability %	54.88	25.08	11.97	7.494	0.48	0.08
Cumulative %	54.88	79.96	91.94	99.42	99.91	100

In group 1 SW, HC, and SV are close to each other which suggests that these variables show similar behavior in terms of their variability and inheritance (Figure 3). This aligns with the interpretation that these traits tend to vary together across different genotypes. Genotypes 6 and 10 in group 1 showed similar trend for the aforementioned traits. In group 2, HI and SI are placed together indicating their relevance, consisting two genotypes (56 and 45) that tend to perform alike for these parameters while SC in group 3 was not found to be related to any other trait in the biplot (Figure 3).

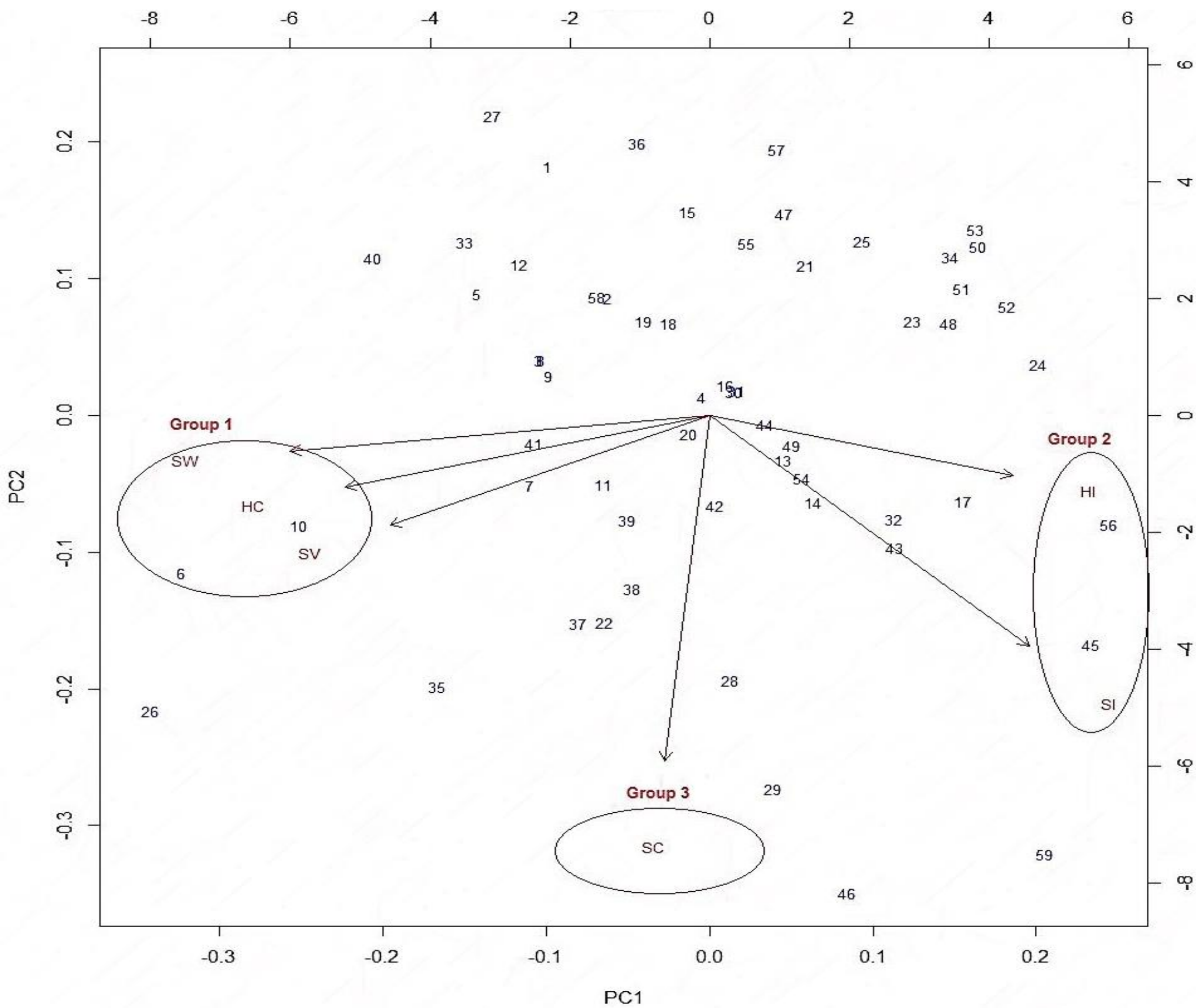


Figure 3. Biplot analysis of various traits in lentil accessions.
HSW=100-seed weight, SV=Seed volume, HC=Hydration capacity, HI=Hydration index, SC=welling capacity and SI=Swelling index

The random scattering of most genotypes suggests that they exhibit diverse patterns across the measured traits. This could indicate that these genotypes have unique combinations of traits that are not strongly correlated with each other. A group of genotypes gathered at the center of the plot may indicate that these genotypes have average to intermediate values for the measured traits. They neither align strongly with the group of genotypes closer to SW, HC, and SV nor with those exhibiting unique patterns. This approach underscores the strategic importance of genotype selection in hybridization breeding programs and emphasizes the integration of trait diversity and stability considerations with breeding objectives to optimize genetic gain.

DISCUSSION

The most significant tools for defining the selection criteria for increased seed production is use of genetic diversity, heritability, and their association. Therefore, it is imperative to identify the positive correlations among growth and yield-related characteristics and to eliminate any negative correlations that may be caused by interactions appeared over time (Tambal et al., 2016). Seed physical attributes and the physiochemical properties are essential to be examined for selecting the best screening quality characters (Santos et al., 2018).

Genetic variability plays key role for a breeder in the selection of the genotypes comprising high yield along with excellent quality traits. Plant breeders are interested mainly in traits having high heritability since they result in greater advance under selection. The values reported for seed weight of lentil were 62.8 %, 87.0%, 91.0%, 98.0%, and 99.0% (Ninou et al., 2019).

This higher heritability for seed weight corresponds to the higher hydration capacity, seed volume and swelling index as indicated by positive correlation coefficient among these studies traits. Our findings are in accordance with the previous studies (Malik et al., 2011; Kumar et al., 2016; Ninou et al., 2019). Several other crops such as cotton, Pea, bean and pigeon pea have also been examined for different physical and physiochemical traits such as 100 seed weight, seed volume, hydration capacity and porosity percent (Taleb et al., 2016).

Genotypic and phenotypic traits can estimate the divergence but the heritable section regarding variation can be determined by the estimation of the heritability extent. In the present study, influence of environmental factors was also recorded on all parameters. The difference between the GCV% and PCV% values for the traits as 100-seed weight, hydration index and hydration capacity indicated the maximum environmental influence on the performance of genotypes. Genetic parameters are the important attributes for heritability information (Kahirizi et al., 2010).

Since extensive heritability, variance and genetic advance, improvement could be projected by selection for number of seeds/ plant and seed yield/plant (Hamdi et al., 2003). High phenotypic differences were observed for number of seeds in lentil. For the characteristics, grain yield and thousands grain weight strong broad sense heritability as well as elevated predicted genetic advancement as percent of mean were recorded. These traits may be attributed to the action of additive genes and could be improved by modifying selecting without progeny testing. They can be observed as advantageous traits for improvement through selection. By using genetic variation present in both exotic genotypes and elite genotypes, the production of the crop can be uplifted (Ghimire et al., 2019).

According to Iqbal et al. (2006) the seed size is associated with hydration and swelling capacities directly. Malik et al. (2011) also found a positive correlation of seed volume and hydration capacity in chickpea which indicated that there was an obvious increase in seed volume after they were soaked overnight. It was concluded that the seed weight has positive significant correlation of with hydration capacity, swelling capacity and seed volume. These results are in accordance to our findings. Similarly, it was further corroborated that the hydration index and swelling capacity are negatively associated (Malik et al., 2011). On other hand, the rate of water uptake can be altered under varying soaking time and temperature in lentil (Joshi et al., 2010). This indicates that the temperature and soaking time also affect the imbibition rate of water in addition to the seed size and weight. However, our study demonstrated the genetic variation for different exotic and indigenous genotypes at 24 hours of soaking time. Likewise, several studies focusing hydration kinetics have corroborated the impact of presoaking and water uptake on the physiochemical properties and cooking qualities in chickpea and lentil (Gowen et al., 2007; Kumar et al., 2016). In crux, these results suggested the studied genotypes/accessions displayed wide range of genotypic diversity and their potential to be used as source for improving the quality traits of lentil in future breeding is promising.

CONCLUSION

In this study, among all the observed lentil genotypes ILL4402×ILL5588 showed most significant results in all the traits as HSW, HC, HI, SI and SV except SC which was found maximum in MANSEHRA 89. ILL10750×ILL1959, FLIP97-34L×FLIP97-33L, L-4147×ILL4649 and ILL4605×ILL6024 appeared promising in all traits respectively, on the basis of their physiochemical attributes. Selection for a valuable germplasm with higher rate of water uptake and desired seed size and weight can potentially improve the cooking and quality traits in lentil genotypes.

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AUTHOR CONTRIBUTIONS

TA and RAS recorded the data, performed experiment and wrote the manuscript. MJ and SRM generate the idea and reviewed the manuscript. SAA and IA reviewed and proofread the manuscript. RAS performed data analysis and visualization.

COMPETING OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research paper. The research was conducted objectively and without any external financial or personal influences that could have affected the findings or interpretation of the results.