

Estimating genetic variability among diverse lentil collections through novel multivariate techniques

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Abstract

Lentil is a significant legume crop throughout the world where Pakistan stands at 18th position with 8610 tons from 17457 hectares. It is nutritionally rich in protein, carbohydrates, mineral, fat and fiber that can potentially meet food security resolving malnutrition issues. Lentil 220 collections representing Pakistan 184, Syria 14, USA 22 and 04 of unknown origin were studied for high yield, yield contributing traits and early cooking time. Results revealed high variability, 6122 (Pakistan) was considered as best during both years due to its performance as seed yield (68 ± 1.7), biological yield (264 ± 2.8), pod size (0.61 ± 0.01), seed per pod (2), cooking time (11 minutes) and hard seed (0). Comparatively 6122 (Pakistan) and 6042 (Syria) produced the highest biological yield which could also be an efficient source for fodder. 5698 (Pak) and 6015 (USA) took 161–172 days in 2018 while 24783 (unknown) and 5561 (Pak) were matured early in 2019. Lowest cooking time 10 minutes was observed in 6074 and 5745 of Pakistani origin. Lentil with the lowest cooking time saves energy and resources, develops adequate flavor, texture and improves protein digestibility. Pearson correlation matrix, Principal component analysis, structural equation model, cluster analysis, heritability showed promising results. In conclusion 12 genotypes were selected on high yield potential and recommended for lentils breeding including 5664, 5687, 6084, 6062, 6122, 6058, 6087, 5689, 6042, 6074, Markaz 2009 & Punjab 2009.

Introduction

Lentil (*Lens culinaris* Medik) is an annual, herbaceous, cool-season, autogamous (self-pollinated) and diploid legume with 4 Gbp genome size^{1,2}. It is domesticated with wheat and barley. One of the oldest dry legumes, about 9000 years ago from wild ancestors that initiated in the Near East and subsequently spread throughout Mediterranean Basin and Central Asia³. Lentil is the 4th crop in the world after bean, pea, and chickpea included seven taxa and four species that are *L. culinaris*, *L. nigricans*, *L. ervoides*, and *L. lamottei*, having two varietal types as macro-sperma (large seeded) and micro-sperma (small-seeded)^{4,5}.

Lentils are consumed due to its third-highest level of protein contents, vitamins, and micronutrients that are a vital part of human nutrition while vegetative parts are being used as fodder for animals⁶. Protein found in lentil seed is of high quality which is about 22 to 35% of total seed mass and consists of a large number of main amino acids like lysine and isoleucine. Moreover, protein found in lentils is a source of low-cost protein in many regions of the world which is a usable source for human beings as well as animals⁷.

Along proteins lentils are good source of carbohydrates (59%), minerals (2%), and has very little fat and fiber playing a significant role to overcome malnutrition issues and deficiency of micronutrients in developing regions⁸. It is also the best source of iron, so it helps prevent iron deficiency⁹. Medicinally, it has low glycemic level so physicians strongly suggest for patients of diabetes, obesity and heart disorders⁹. Seeds of lentils are mucilaginous and laxative recommended for treating constipation, stomach infections, smallpox and ulcer⁶. Top ten lentil producing countries are Canada, India, Turkey, USA, Kazakhstan, Nepal, Australia, Russia, Bangladesh, and China¹⁰.

The imperative causes of low yield of lentil in Pakistan are cultivation on marginal lands with poor management practices, cultivation of local varieties, biotic and abiotic factors but the worse is adaptation of low yielding local genotypes in different environments due to low genetic variability¹¹. For combating effects of biotic and abiotic stresses by application of herbicides, pesticides, and fertilizer as well as by improving irrigation systems are not cautiously practicable¹². Best solution to this problem is to make superior planting material. In this regard plant breeding and modern biotechniques could improve crops by transferring a gene of interest. The insufficient information of genetic variability in existing lentil is major hindrance to getting highest potential of the crop and modern techniques as well¹³. Considering this, current studies were conducted to determine variation in agro-morphological traits among genotypes representing Pakistan, USA, and Syria.

Assessment of genetic diversity based on phenotypic markers has been conducted worldwide in the near past in lentil and other legumes^{3,7,11,14–18}. Germplasm collections are valuable assets that possess a huge treasure of genetic material which can prove its worth in future crop improvement and development programs¹⁹. Genetic diversity can be estimated with the help of quantitative traits and qualitative as well²⁰. A large number of morphometric techniques have been used to measure and classify genotypes based on genetic diversity as in pea, mungbean, alfalfa, black gram, soybean, and lentil in the near past. Likewise, current studies also used various statistical techniques pursuing the genetic diversity in the available genetic resources. This study was designed to evaluate diverse lentil collections for promising traits concerning qualitative and quantitative for consecutively two years focusing to identify novel material for starting lentil breeding program.

Results

Descriptive statistics for agro-morphological traits

Quantitative and qualitative descriptive statistics were performed for evaluation and characterization of lentil genotypes grown in 2018 and 2019. Mean, variances, standard error of mean were analyzed for quantitative traits while frequency distribution was determined for qualitative traits. Results revealed that genotypes 6122 (Sheikhupura, Pak) was considered as the best genotypes based on quantitative traits. It performed best for 6 quantitative traits out of 12 that are Seed yield (68 ± 1.7), biological yield (264 ± 2.8), pod size (0.61 ± 0.01), seed per pod (2), cooking time (11 minutes), Hard seed (0). Descriptive statistics for quantitative traits of lentil genotypes showed that mean \pm SE values of seed yield (30.08 ± 0.98 g) were higher in 2018 as compared to seed yield (25.26 ± 0.87 g) in 2019 (Table 1). Check varieties showed reduced seed yield in 2019 as compared to yield of 2018.

Seed yield ranged from 5.0 to 85.00 g and 3.00 to 76.00 g in 2018 and 2019 respectively. Genotype 6084 (Narowal, Pak) depicted highest seed yield in 2018 (85 ± 0.07 g) in 2019 (76 ± 2.9 g). Whereas lowest seed yield (5 ± 0.5 g) was noted in genotype 5475 (Gujranwala, Pak) in 2018 while during 2019 lowest seed yield (3 ± 0.3 g) was noted in 5494 (Okara, Pak) and 5475 (Gujranwala, Pak). Genotypes 6052 (Syria), 5583 (Muzaffargarh, Pak) and 5556 (Hyderabad, Pak)

produced highest hundred seed weight 3.65 g, 3.64 g and 2.97 g respectively in 2018 whereas during 2019 genotypes 5583 (Muzaffargarh, Pak), 6052 (Syria) and 5684 (Narowal, Pak) produced 3.28 g, 3.2 g and 2.71 g hundred seed weight, respectively.

Table 1.
Descriptive statistics for quantitative traits of lentil grown in 2018 and 2019.

During 2018							During 2019					
Traits	Mean	Min.	Max	V (n)	SD	VC(n)	Mean	Min.	Max.	V (n)	SD	VC(n)
SY	30.08 ± 0.9	5	85	211	14.52	0.48	25.26 ± 0.8	3	76	166.8	12.91	0.51
HSW	1.71 ± 0.02	1.05	3.65	0.12	0.35	0.2	1.68 ± 0.01	1.17	3.28	0.07	0.28	0.16
BY	114.6 ± 3.0	42	264	1977	44.4	0.38	94.13 ± 2.2	34	187	1074	32.77	0.34
PH	44.73 ± 0.4	27.38	71.12	47.08	6.86	0.15	46.7 ± 0.4	29.6	69	48.28	6.94	0.14
LPH	14.97 ± 0.1	9.14	32.16	5.49	2.34	0.15	14.98 ± 0.1	10.72	33.34	4.15	2.03	0.13
PS	0.3 ± 0.0	0.2	0.7	0.007	0.08	0.22	0.3 ± 0.00	0.2	0.7	0	0.08	0.22
SP	2.00 ± 0.0	1	3	0.02	0.16	0.08	2.00 ± 0.01	1	3	0.08	0.28	0.14
MD	184 ± 0.3	161	198	25.5	5.05	0.02	186 ± 0.34	161	198	25.5	5.05	0.02
CT	14.55 ± 0.1	10	18	2.32	1.52	0.1	16.15 ± 0.1	10	18	2.32	1.52	0.1
HS	0.37 ± 0.0	0	2	0.37	0.61	1.65	0.41 ± 0.04	0	2	0.37	0.61	1.65
NP	2.04 ± 0.0	2	3	0.03	0.19	0.09	2.00 ± 0.01	1	3	0.02	0.16	0.08
SY = Seed yield, HSW = Hundred seed weight, BY = Biological yield, PH = Plant height, LPH = Lower pod height, PS = Pod size, MD = Maturity day, CT = Cooking time, HS/hs = Hard seed/hundred seed, NP = Numbers of pod, SP = Seed per pod Standard deviation (n) = SD (n), Variation coefficient (n) = CV, Standard error of mean = SE,												

Mean ± SE of biological yield for all genotypes was 114.6 ± 3.0 g during 2018 while during 2019 it was 94.13 ± 2.0 g. Genotypes 6122 (Sheikhupura, Pak), 5689 (Sialkot, Pak), 5730 (Layyah, Pak), 6037 (Syria) produced highest biological yield 264 ± 2.8 g, 258 ± 1.0 g, 254 ± 1.5 g, and 254 ± 2.0 g respectively in 2018. Whereas genotypes 6042 (Syria), 6087 (Narowal, Pak) and 5689 (Sialkot, Pak) produced highest biological yield i.e. 187 ± 1.3 g, 183 ± 0.9 g and 179 ± 1.3 g respectively in 2019. Mean values ± SE of plant height in 2018 had 44.73 ± 0.46 cm which was less than recorded in 2019 (46.7 ± 0.4 cm). Genotype 5593 (Hyderabad, Pak) depicted highest plant height (71.12 ± 1.7 cm) during 2018 while during 2019 genotype 6062 (Syria) had highest plant height (69.00 ± 2.1 cm). Genotypes originated from Pakistan showed best performance under conditions as compared to germplasm acquired from Syria and USA.

Mean value ± SE of lower pod height during 2018 had 14.97 ± 0.15 cm whereas during 2019 lower pod height had 14.98 ± 0.13 cm. Largest pod size attained a length of about 0.7 ± 0.1 cm during 2018 whereas during 2019 it had 0.7 ± 1.1 cm. Mean values ± SE of seeds per pod were 2.00 ± 0.01 in 2018 and similar results were also recorded in 2019. Genotypes 6074 (Bahawalnagar Pak), 5595 (Kharan, Pak), 6075 (Bahawalnagar, Pak) had 3.00 ± 0.0 seed per pod in 2018 while highest number of seed per pod (3.00 ± 0.0) during 2019 were reflected by genotypes 5550 (Hyderabad, Pak), 5637 (Hyderabad, Pak), 5625 (Muzaffargarh, Pak), 5555 (Hyderabad, Pak), 5677 (Layyah, Pak), 5630 (Rajanpur, Pak), 5538 (Thatta, Pak), 5583 (Muzaffargarh, Pak), 5654 (Mianwali, Pak) and 5518 (Muzaffargarh, Pak). Days to maturity ranged from 161 to 198 days in 2018 and similar results were found in 2019 but mean values of days to maturity were 184 ± 0.34 during 2018 while 186 ± 0.34 during 2019. Highest days to maturity (196 to 198 days) was counted in 5684 (Narowal, Pak), 5988 (USA), 5979 (USA), 5679 (Narowal, Pak), 5982 (Narowal, Pak), 5683 (Narowal, Pak), 24786 (unknown), 5623 (Faisalabad), 6077 (Bahawalnagar, Pak) and 5684 (Narowal, Pak) while earliest maturing (161 to 178 days) genotypes 5698 (Sialkot, Pak), 6015 (USA), 5667 (Layyah, Pak), 5748 (Gujranwala, Pak), 5555 (Hyderabad, Pak), 6017 (USA), 5700 (Sialkot, Pak), 5571 (Jacobabad, Pak), 5580 (Ghotki, Pak), 5981 (USA) were recorded in 2018. Similar genotypes were also shown highest and lowest days to maturity in 2019.

Mean value of hard seeds was 0.37 ± 0.04 in 2018 and 0.41 ± 0.04 in 2019. Highest number of hard seeds noted in 2018 and 2019 was 2. Number of pods per peduncle ranged from 2 to 3 during 2018 and 1 to 3 during 2019. Mean value of the number of pods per peduncle in 2018 were 2.04 ± 0.01 whereas in 2019 was 2.00 ± 0.01. Two check varieties including Markaz 2009 and Punjab 2009 were also analyzed. Descriptive statistics of check varieties revealed that seed yield, hundred seed weight, and biological yield was higher in 2018 (Table 2) as compared to 2019 in both Markaz 2009 and Punjab 2009 varieties. Variance in seed yield (211), hundred seed weight (0.12), biological yield (1977), plant height (47.08), lower pod height (5.49), pod size (0.007), seed per pod (0.02), days to maturity (25.5), cooking time (2.32), hard seed (0.37) and number of pods per peduncle (0.03) were recorded in 2018. Traits with highest variance were biological yield (1977), seed yield (211) and plant height (47.08) in 2018. Whereas lowest variance in 2018 was recorded in pod size (0.007) and seed per pod (0.02). Similarly, biological yield (1074), seed yield (166.8) and plant height (48.28) were considered the highest variant traits in 2019 (Tables 1 and 2).

Table 2

Best genotypes performed during 2018 and 2019 to specific traits. SY = seed yield, HSW = hundred seed weight, BY = biological yield, PH = plant height, LPH = lower pod height, PS = pod size, SP = seed per pod, MD = maturity day, CT = cooking time, HS = hard seed, NP = numbers of pod

List of best genotypes during two years		
Traits	During 2018	During 2019
SY > 40	6084, 6062, 6122, 6058, 6087, 5689, 6042, 6074, 5664, 5687, Markaz 2009, Punjab 2009	6084, 6062, 6122, 6058, 6087, 5689, 6074, 6042, 5664, 5687, Markaz 2009, Punjab 2009
HSW > 1.91	6052, 5583, 5556, 6101, 6124, 5684, 5856, 23776, 6054, 5643, Markaz 2009, Punjab 2009	5583, 6052, 5684, 6062, 6101, 5556, 5643, 5553, 6086, 2377 Markaz 2009, Punjab 2009
BY > 87.5	6122, 5689, 5730, 6037, 6042, 5600, 5653, 5982, 5658, 6084, Markaz 2009, Punjab 2009	6042, 6087, 5689, 6012, 6037, 6052, 6060, 24786, 24783, 6090 Markaz 2009, Punjab 2009
PH > 55.1	5593, 5549, 5694, 5687, 5518, 5472, 5622, 5500, 5506, 5723, Markaz 2009, Punjab 2009	6062, 6067, 6060, 6074, 5485, 5562, 6104, 24783, 5538, 6064, Markaz 2009, Punjab 2009
LPH > 15.6	5527, 6062, 6013, 6041, 6042, 5518, 5686, 5993, 5529, 6002, Markaz 2009, Punjab 2009	6099, 5717, 5695, 6097, 5684, 6101, 6124, 6060, 5693, 5667 Markaz 2009, Punjab 2009
PS > 1.36	6010, 6013, 6041, 23776, 6124, 6122, 6043, 5996, 6059, 6076, Markaz 2009, Punjab 2009	5550, 5556, 5570, 5667, 5665, 5661, 5575, 5531, 5628, 5623, Markaz 2009, Punjab 2009
SP > 2	6074, 5595, 6075, 6010, 6013, 6041, 23776, 6124, 6122, 6043, Markaz 2009, Punjab 2009	5550, 5637, 5625, 5555, 5677, 5630, 5538, 5583, 5654, 5518, Markaz 2009, Punjab 2009
DM < 170	5698, 6015, 5667, 5748, 5555, 6017, 5700, 5571, 5580, 5981, Markaz 2009, Punjab 2009	6092, 6099, 5511, 5518, 5517, 6097, 6014, 5628, 5677, 6082, Markaz 2009, Punjab 2009
CT < 12.09	6074, 5595, 6075, 6010, 6013, 6041, 23776, 6124, 6122, 6043, Markaz 2009, Punjab 2009	6092, 6099, 5511, 5518, 5517, 6097, 6014, 5628, 5677, 6082, Markaz 2009, Punjab 2009
HS < 2	6074, 5595, 6075, 6010, 6041, 23776, 6124, 6122, 6043, 5996, Markaz 2009, Punjab 2009	5529, 5555, 5636, 5485, 5553, 5861, 5677, 5643, 5856, 5650, Markaz 2009, Punjab 2009
NP > 2	5995, 5861, 5691, 5671, 6045, 5689, 5537, 5685, 6047, 6074, Markaz 2009, Punjab 2009	5595, 6075, 6074, 5480, 5532, 23779, 5742, 5637, 5700, 5694, Markaz 2009, Punjab 2009

Phenotypic traits data analysis

Ground color of testa exhibit four categories out of which grey color showed highest frequency distribution (51.82%) while brown (33.64%), black (13.19%) and green (1.37%) ranked as second, third and fourth respectively during both years (2018 and 2019). Cotyledon color had the highest frequency (96.37%) of orange/ red color out of three categories during both years. Color of pattern on testa had five categories. Highest frequency (59.54%) was recorded for category 2 (grey). Pod dehiscence was very low during both years. About 89.10% of lentil genotypes had low pod dehiscence frequency while high pod dehiscence was observed only in 0.90% genotypes. Other qualitative traits with frequency distribution are given in Table 3.

Correlation Matrix (Pearson)

Correlation matrix for 11 quantitative traits of lentil genotypes grown in 2018 revealed that seed yield showed a significant positive correlation with biological yield (0.73*), pod size (0.32*), seeds per pod (1.50*) and number of pods per peduncles (0.18*), as shown in Table 4. Hundred seed weight exhibits a significant positive correlation with pod size (0.29*). Similarly, biological yield also showed a positive correlation with pod size (0.34*). Whereas plant height and pod size showed a significant positive correlation with seed per pod (0.17*) and maturity days (0.15*) respectively. Whereas lentil genotypes grown in 2019 indicated that seed yield exhibits a significant positive correlation with a hundred seed yield (0.27*), biological yield (0.83*), plant height (0.21*). Hundred seed weight also showed a positive correlation with biological yield (0.31*), similarly, biological yield showed a positive correlation with plant height (0.16). Positive combinations and coordination describes high potential of the germplasm for its utilization in future crop improvement programs.

Table 3
Frequency distribution of qualitative traits of lentil during 2018 and 2019

Variable	No. of categories	Categories Name	Frequency (%)	Variable	No. of categories	Categories name	Frequency (%)
Ground color of testa	4	Green	1.36	Pod shedding	3	Low	59.54
		Grey	51.81			Medium	27.27
		Brown	33.63			High	13.18
		Black	13.18	Tendrill length	1	Prominent	100
Cotyledon color	3	Yellow	2.27	Ground color of flower	1	White blue lines	100
		Orange/red	96.36	Leaflet size	3	Small	62.72
		Olive-green	1.36			Medium	28.18
Color of pattern on testa	5	Absent	0.90			Large	9.09
		Olive	7.72	Pod pigmentation	2	Absent	69.09
		Grey	59.54			Present	30.90
		Brown	13.63	Leaf pubescence	2	Slight	85
Pod dehiscence	4	Black	18.18			Dense	15
		None	0.90	Seedling stem pigmentation	2	Absent	69.09
		Low	89.09			Present	30.91
		Medium	9.09				
		High	0.90				

Table 4.
Correlation matrix (Pearson) for quantitative traits of lentil grown in 2018 and 2019.

Traits	Years	HSW	BY	PH	LPH	PS	SP	MD	CT	HS	NP
SY	2018	0.09	0.73*	-0.09	0.03	0.32*	0.15*	0.01	-0.05	0.11	0.18*
	2019	0.27*	0.83*	0.21*	-0.11	-0.11	-0.11	-0.05	-0.19*	-0.01	0.08
HSW	2018		0.06	-0.13	-0.05	0.29*	-0.04	0.05	0.04	-0.09	-0.01
	2019		0.31*	0.05	-0.06	-0.04	0.03	-0.05	-0.01	-0.03	-0.05
BY	2018			-0.02	-0.04	0.34*	0.03	0.03	-0.02	0.06	0.13
	2019			0.16*	-0.16*	-0.10	-0.08	-0.08	-0.08	-0.04	0.06
PH	2018				0.03	-0.12	0.17*	-0.04	0.06	0.06	-0.06
	2019				0.12	0.05	0.01	-0.12	-0.05	0.06	0.05
LPH	2018					-0.07	-0.02	-0.09	0.03	-0.06	0.02
	2019					-0.03	-0.00	0.11	-0.05	0.05	-0.05
PS	2018						0.00	0.15*	0.01	-0.06	-0.06
	2019						0.10	-0.00	0.10	-0.04	-0.09
SP	2018							0.00	0.07	-0.04	0.00
	2019							0.09	0.18	-0.02	0.00
DM	2018								0.00	-0.04	0.03
	2019								-0.08	-0.08	0.00
CT	2018									-0.04	0.03
	2019									-0.13*	-0.13
HS	2018										0.06
	2019										0.00
SY = seed yield, HSW = hundred seed weight, BY = biological yield, PH = plant height, LPH = lower pod height, PS = pod size, SP = seed per pod, MD = maturity day, CT = cooking time, HS = hard seed, NP = numbers of pod											

Principal Component Analysis (PCA)

Principal component analysis based on the performance of traits studied in 2018 indicated that first five components have more than one eigenvalue such as 2.09, 1.37, 1.19, 1.08, 1.02 for F1, F2, F3, F4, and F5 respectively, which have cumulative percentage up to 19.03, 31.54, 42.42, 52.33 and 61.67 (Table 5). Whereas other Principal components (F6 to F10) show less than 1 eigen value. Contribution of variables indicated that seed yield (35.15) biological yield (33.84) and pod size (19.37) represent highest F1 values whereas in F2 highest values were shown by hundred seed weight (23.41) plant height (15.62), and hard seed (13.26). Diverse genotypes have been divided into components that reflect PCA as an artificial tool for further selection of characters based on genetic variability. PCA indicated that the F1 component showed 19.05% total variation while F2 showed 12.51% total variation. Factors loading in F1 showed seed yield (0.84), biological yield (0.84), and number of pods per peduncle (0.02) had highest values. While the F2 component showed highest factor loading with highest positive values for hundred seed weight (0.32), pod size (0.63) and maturity days (0.15). Total variation between F1 and F2 components was observed on PCA was 31.54% (Fig. 1). Selection can be made for individual as well as a whole principal component for improvement of crop.

Scattered diagram obtained from PCA analysis during 2018 indicated that lentil was classified into two factors i.e F1 and F2 (Fig. 2). F1 contributed 19.04% variability and F2 indicated 12.19% variability. Following genotypes (5638, 5583, 6124, 6052, 6010, 5684, 5979, 6101) were noted to be present in the extreme upper left side of the scattered plot. Genotypes (6054, 5556, 23776, 5650) were placed at an extreme of the upper right side. Similarly, on the lower left side of scattered plot genotypes 6122, 6084, 5689, 5590 were present. Likewise, few genotypes viz., 5595, 5593, 5518 5549 showed their presence at extreme lower right side. Material with distinct output could be used for new combinations during breeding are suggested.

Similarly, principal component analysis of lentil genotypes grown in 2019 indicated that first five components have more than one eigen value such as 2.19, 1.34, 1.15, 1.11, 1.03 for F1, F2, F3, F4, and F5 respectively, having cumulative percentage up to 19.91%, 32.10%, 42.62%, 52.78%, and 62.67% respectively (Table 6). Remaining principal components (F6 to F10) show less than 1 eigen value. Contribution of variables indicated that SY (37.46) BY (36.51) and HSW (9.87) represent highest F1 values whereas in F2 highest values were shown by CT (31.77) SP (14.37) and HS (12.19). Separation of genotypes and their accumulation in the particular principal component indicates divergence like yield and yield contributing traits towards one PC that proves PCA a tool for further selection of characters for handling and crop improvement. PCA indicated that the F1 component showed 19.91% total variation while F2 showed 12.19% total variation. Total variation between F1 and F2 components was observed on PCA 32.10% (Fig. 3).

Table 5
Principal component analysis for quantitative traits of lentil studied during 2018

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Eigenvalue	2.09	1.37	1.19	1.08	1.02	0.95	0.89	0.80	0.76	0.57	0.23
Variability (%)	19.03	12.50	10.88	9.90	9.34	8.63	8.09	7.31	6.91	5.19	2.17
Cumulative %	19.03	31.54	42.42	52.33	61.67	70.30	78.39	85.71	92.62	97.82	100
Contribution of variables (%)											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
SY	35.15	6.56	0.06	0.56	0.50	0.24	0.03	0.69	0.20	5.30	50.65
HSW	4.89	23.41	1.73	2.17	0.00	10.01	2.33	41.90	0.48	12.89	0.15
BY	33.84	4.56	0.02	0.02	0.87	0.01	0.55	4.91	3.89	6.65	44.65
PH	2.07	15.62	20.24	4.11	2.06	0.53	7.19	8.68	38.17	0.24	1.05
LPH	0.33	3.01	0.00	51.58	2.12	4.49	25.60	6.59	5.44	0.24	0.56
PS	19.37	11.21	2.96	0.27	2.64	0.91	2.42	0.00	0.00	60.18	0.01
SP	0.45	8.64	35.05	0.88	0.01	5.98	17.71	4.00	24.71	0.44	2.09
MD	1.10	7.98	2.06	16.08	16.97	20.89	28.48	0.43	1.71	4.22	0.03
CT	0.11	0.00	19.90	6.22	23.84	30.67	2.95	14.79	0.96	0.40	0.12
HS	0.25	13.26	11.02	15.05	0.02	24.41	7.63	9.61	17.83	0.39	0.50
NP	2.40	5.70	6.94	3.03	50.93	1.83	5.06	8.37	6.56	8.99	0.15

Table 6
Principal Component Analysis (PCA) for quantitative traits studied during 2019

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
Eigenvalue	2.19	1.34	1.15	1.11	1.03	0.95	0.92	0.78	0.69	0.65	0.15
Variability (%)	19.91	12.19	10.52	10.16	9.37	8.65	8.41	7.13	6.30	5.90	1.42
Cumulative %	19.91	32.10	42.62	52.78	62.15	70.81	79.22	86.36	92.66	98.57	100
Contribution of variables (%)											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
SY	37.46	0.18	0.02	1.52	0.00	0.15	0.62	5.78	1.65	2.67	49.91
HSW	9.87	7.68	0.00	1.89	6.70	10.36	1.16	56.41	0.00	5.80	0.07
BY	36.51	2.26	0.20	0.43	0.02	0.03	0.09	6.02	3.70	1.79	48.90
PH	4.26	0.57	41.65	3.35	6.62	6.10	1.17	0.42	14.59	21.09	0.13
LPH	1.85	9.83	11.96	27.26	5.26	1.94	11.24	1.62	15.00	13.83	0.10
PS	2.12	11.95	9.81	0.45	1.20	6.99	53.47	4.28	8.67	1.03	0.00
SP	1.93	14.37	1.09	13.19	17.28	26.95	0.44	1.27	8.20	15.23	0.00
MD	1.23	1.127	16.04	46.05	0.10	0.62	6.80	3.87	0.65	23.43	0.04
CT	3.54	31.77	0.97	0.95	0.06	0.02	17.92	7.31	24.94	11.64	0.83
HS	0.01	12.90	14.35	4.65	0.39	46.69	5.62	2.99	9.28	3.08	0.00
NP	1.16	7.32	3.86	0.23	62.32	0.11	1.43	9.97	13.21	0.36	0.00

Genotypes at extreme of upper left side of the scattered plot including (5506, 5729, 5510, 5672, 5636, 5581, 5491, 5483) while in lower left side genotypes (5472, 5512, 5479, 5486, 5650, 5600, 5531) were placed at a lower extreme which showed wide variation in genotypes. Similarly, at upper right side of scattered plot, genotypes (6080, 6043, 5653, 6043, 6002, 6060, and 5677) showed at extreme point while in lower right-side genotypes 23787, 24787, 23779, 5742, 5712 were placed at extreme variability point (Fig. 4).

Hierarchical cluster analysis

The cluster analysis based on agro-morphological data (obtained in 2018) distributed 220 lentil genotypes into 4 main clusters at linkage distance (LD) of 14.5 (Fig. 5). Cluster C1 further subdivided at LD 9.4 into two sub-cluster i.e C2a and C2b. C2a have only one genotype (5698 Sialkot, Pak) while C2b further subdivided into two sub-clusters i.e., C2bi and C2bii. C2bi consisted of 32 genotypes whereas C2bii consisted of 51 genotypes. Cluster C1 further subdivided into two sub clusters i.e., C1a and C1b. C1a consist of only one genotype (6015 USA) while C1b further subdivided into C1bi having 32 genotypes and C1bii having 64 genotypes at LD 4.4. Cluster C3 consisted of two sub-clusters C3a and C3b at LD 9.4. Sub-cluster C3a consist of only 3 genotypes (5689 Sialkot, 5687 Narowal, 6084 Narowal) belonged to Pakistan while sub-cluster C3b further subdivided into C3bi and C3bii with 10 and 6 genotypes respectively. Cluster C4 also subdivided into two sub-clusters one is C4a having 6 genotypes and other is C4b with 3 genotypes at LD 9.4. Highest number of genotypes were found in cluster 1 (115genotypes) While C4 has lowest number of genotypes (9 genotypes) in 2018. The hierarchical dendrogram obtained from lentil agro-morphological data (2019) indicated 7 main clusters named as C1, C2, C3, C4, C5, C6, and C7 at 50% LD (8.4) (Fig. 6). Cluster C1 consisted of 37 genotypes while cluster C2 subdivided into two sub-clusters C2a and C2b. C2a consisted of 35 genotypes while C2b further subdivided into C2bi and C2bii. Sub-cluster C2bi consisted of 35 genotypes while sub-cluster C2bii consisted of only 4 genotypes (6099 Rawalpindi, 6092 Rawalpindi, 5511 Muzaffargarh, 5517 Muzaffargarh) of Pakistan origin. Cluster C4 consisted of only 12 genotypes whereas cluster C5 and C6 consisted of 1 (24783 of unknown origin) and 4 genotypes (6084 Narowal-Pak, 5056 Syria, 6062 Syria, and 6122 Sheikhpura-Pakistan) respectively. Cluster C6 consists of two sub-cluster i.e C6a and C6b with 17 and 32 genotypes. Cluster C7 consisted of only 2 genotypes (5664 Layyah and 6074 Bhawalnager) belonged to Pakistan.

Structural Equational Model (SEM)

Structural Equational Model (SEM) was built for seed yield (SY) based on covariance study as shown in Table 7. Seed yield was taken as a dependent variable whereas other traits such as SW, BY, PH, LPH, PS, MD, CT, HC, NP, and NSP were taken as independent variables (Fig. 7). Contribution of independent variables like LPH (P-values = 0.76), PS (P-values = 0.91), MD (P-values = 0.66), CT (P-values = 0.70), HC (P-values = 0.98), NP (P-values = 0.94), NSP (P-values = 0.93) observed significant (significant level < 1) for yield as dependent variable which showed that relationship is perfect as indicated via SEM. This was further verified by lowest error variance of multiple linear regressions. Value of coefficient of determination ($R^2 = 0.683$) of this multiple linear relationship supports this hypothesis that real yield contributors are given in equation.

Heritability analysis

Heritability is a statistical tool that estimates the degree of variation in phenotypic traits in a population based on genetic variation. Current studies revealed that heritability ranged from 0 to 1. Heritability was highest for pod pigmentation (1), leaf pubescence (1), seedling stem pigmentation (1), leaf size (0.99), hundred seed weight (0.93), seed yield (0.87), biological yield (0.72), pod size (0.64), pod shedding 0.64 while heritability was low in cooking time 0.9, and ground color of testa 0.17, while remaining traits had zero heritability. Values less than 1.00 elaborated that genes do not contribute at all to phenotypic individual differences whereas heritability values 1.00 mean genes are the only reason for individual differences (Table 8).

Discussion

Selection of best genotypes is always a thrusting search by scientists to develop cultivars for farmer's fields. Agro-morphological diversity has been used to characterize the genotypes in a range of plant species including lentil²¹. Wide variation was observed in lentil seed yield from 3–85 g with genotype 6084 Narowal (Pak), highest seed yield in 2018 (85 ± 0.07 g) and 2019 (76 ± 2.9 g) while previous studies showed 9.65 g maximum seed yield after studying 197 lentil genotypes¹⁶. Seed yield potential and variability offer breeders to develop high yielding cultivars with adopting varying environments²². Biological yield increases the photosynthetic area of the plants which enhance food quality and seed yield. In our study Genotypes 6122 Sheikhpura, 5689 Sialkot), 5730 (Layyah) of Pakistan origin and 6037 exhibited best biological yield during 2018. While genotypes 6042 (Syria), 6087 (Narowal, Pak) and 5689 (Sialkot, Pak) indicated best biological yield in 2019. Biological yield produced during 2018 and 2019 was higher and performed best ($42\text{--}264$ g/plant) as compared to previous studies biological yield ranged were ($2.04\text{--}19.22$ g/plant)¹⁶ ($5.43\text{--}40.07$ g)¹⁷. (87.38 g)²³. Genetic variability in biological yield had a significant role in breeding program²⁴. The improvement in biomass at the vegetative growth stage prior to flowering, leads to further increase in N-assimilation and thereby in seed yield^{25,26}.

Table 7.
Structural Equations of model

SY	SW	BY	PH	LPH	PS	MD	CT	HC	NP	NSP
Stander	9606.03	9601.04	19202.89	1.89	202.65	0.10	2.68	18.19	30.82	21.85
Z-values	0.00	0.00	0.000	0.31	0.11	0.43	0.39	0.02	0.07	0.08
P-values	1	1	1	0.76	0.91	0.66	0.70	0.98	0.94	0.93
SY = 2.099*SW + 0.162*BY + 0.135*PH + 0.576*LPH + 21.789*PS – 0.0442*MD – 1.038*CT + 0.362*HC + 2.298*NP + 1.826*NSP										

Table 8
Heritability analysis of traits quantitative and average performance of check varieties in divers 220 Lentil genotypes during 2018 and 2019

Variable	V _G	V _P	h ² _{bs}
Seed yield	147.43	168.60	0.87
Hundred seed weight	0.0902	0.09	0.93
Biological yield	872.64	1,203	0.72
Plant height	0.00	26.91	0.00
Lower pod height	0.00	2.55	0.00
Pod size	2.037	3.15	0.64
Seed per pod	0.00	0.02	0.00
Maturity days	0.00	13.20	0.00
Cooking time	0.11	1.22	0.90
Hard seed	0.00	0.21	0.00
Number of pods/peduncles	0.00	0.01	0.00
Ground color of testa	0.0519	0.2992	0.17
Cotyledon color	0.0000	0.5371	0.00
Testa pattern color	0.0000	0.2139	0.00
Pod dehiscence	0.0320	0.3596	0.89
Pod shedding	2.0370	3.1570	0.64
Tendrill length	0.0000	0.0000	0.0
Flower ground color	0.0000	0.0000	0.0
Leaf size	1.7103	1.7193	0.99
Pod pigmentation	0.2145	0.2145	1.00
Leaf pubescence	2.0493	2.0493	1.00
Seedling stem pigmentation	0.2145	0.2145	1.00

Seed yield and biological yield were higher in 2018 as compared to 2019 that probably due to temperature variation in both years. Average temperature was 21°C in March and 26°C in April during 2018 when the crop was getting mature while temperature was 19°C in March and 24°C in April during 2019. Literature showed that optimum temperature for best lentil yield is 31.5°C. Low temperature played significant role as reducing flowering and pod filling process and resultantly delay maturity. Lentil requires low temperature during vegetative stage, while the reproductive stage needs high temperatures²⁷. Optimum temperature for vegetative stage of lentil ranged from 6.1 to 27.3°C while 17.4 to 31.5°C during reproductive stage²⁸. Genotype 5593 Hyderabad, Pak was recorded with highest plant height in 2018 (71.12 cm) while in 2019 genotype 6062 (Syria) had highest plant height (69 cm). Plant height of lentil reported in 2017 was ranged from 25.93-35.70 cm²³ which is lower than recorded in current studies. Another study reported similar findings like 33.02 to 76.20 cm during 2018¹⁶. Plant height is a major yield contributing trait which support sufficient vegetative growth and branching provides an opportunity that ultimately develop sufficient number of pods and high contribution toward increased seed yield^{7, 29}. Time of maturity is considered as an important trait with its effective mechanism against drought, early maturity and seed set before onset of terminal drought is an important trait in grain legumes. In our study 5698 (Sialkot, Pak) and 6015 (USA), were considered as early maturing genotype with 161-172 days in 2018 while 24783 (unknown) and 5561 (Sanghar, Pak) were found early maturing 161-172 days during 2019 which is more appealing as compared to previous reports of 168-190 days. Development of early maturing cultivars is one of the breeding targets generally desired against drought in crops and in lentils, particularly³⁰.

It is evident that seed yield is directly proportional to number of seeds per pod, higher the seed per pod higher will be seed yield. During current studies number of pods per peduncle ranged from 2-3 in 2018 and 1-3 in 2019. A constant variation in the number of pods per peduncle under different environmental conditions was also reported by various authors¹⁷.

Hundred seed weight is used as an indicator of seed quality and is a measure of grain bulk density. Our study showed that the highest hundred seed weight was 3.65g in 2018 and 3.28 g in 2019, coupled with a wide variation in both years. It was also noted that hundred seed weight was low in 2019 as compared to 2018. Similar studies were also performed by various researchers to characterize and evaluate a wide range of crops including lentil^{3, 7, 11, 14-18}.

Another aspect of lentils is lowest cooking time considered a favorite trait for consumers. In the current studies, lowest cooking time 10 min has been observed in genotypes 6074 (Bahawalnagar, Pak) and 5745 (Faisalabad, Pak). Lentil with the lowest cooking time for grains saves energy and time, develops adequate flavor, texture and improves protein digestibility. While the highest cooking time causes proteins, vitamins, minerals and other secondary metabolites loss. Results showed that genotype 6122 (Sheikhupura, Pak) performed best out of 220 lentil genotypes based on quantitative traits. Therefore, suggested for further trials and breeding program for varietal development.

Grey ground color of testa and orange-red cotyledon color with grey color of pattern on testa was found in most genotypes. Various markets demand specific seed coat colors and/or patterns. We attempted to determine the percentage of color of testa, cotyledon color and color of pattern. Inheritance studies involving crosses between parental lines with various seed coat colors and patterns indicated that seed coat color is determined by two independent loci³¹. Pod pigmentation was absent in 69 % genotypes with low Pod dehiscence and shedding. Low Pod dehiscence and shedding are considered most favorite traits in farmers perspective because low pod dehiscence and shedding reduce grain loss during harvesting. Slight leaf pubescence with a small leaf and prominent tendril was most frequently found. While dense pubescence only found in 15 % of genotypes. Presence of dense leaf pubescence (leaf hairs) reduces absorbance of photosynthetically active radiation (400 to 700 nanometers). Reduction in photosynthetic rate is caused by decreased light absorption rather than decreased carbon dioxide conductance through boundary layer that is supported by Mohammed et al.,¹⁷ who described that all studied genotypes had prominent tendril. It has been also reported that light leaf pubescence with a small leaf and prominent tendril exhibited high resistance to lodging. Furthermore, Stem pigmentation was present in most varieties. All genotypes exhibit white blue line flower³². Seedling stem pigmentation was absent in 69 % of genotypes.

Improvement of a target character can be achieved by indirect selection via other characters that are more heritable and easier to select³³. This strategy of selection requires understanding interrelationships among characters and with target character. A significant correlation between seed yield and most agro-morphological traits was documented in both years 2018 and 2019. Seed yield showed a significant positive correlation with biological yield, pod size, seed per pod and number of pods per peduncles. Hundred seed weight exhibits a significant positive correlation with pod size in 2018 while in 2019 seed yield exhibits a significant positive correlation with a hundred seed yield, biological yield, and plant height. Seed yield per ha was mainly associated with increase in percentage of seed germination, pods per plant and seed index, while prolonged flowering and maturity of lentil crop reduces yield^{34,35}. Number of pods per plant has a significant positive correlation with yield and yield contributing traits like biological yield & hundred seed weight^{16,36}.

PCA is an approach used statistically to distribute number of variables in data by extracting important one from a wide number of genotypes. Study showed that the first 5 components had eigenvalue >1. Contribution of variables toward traits revealed that seed yield, biological yield, and pod size had highest values in F1 which represent high variability among these traits whereas in F2 highest values were recorded in a hundred seed weight, plant height, and hard seed which are recommended for their future utilization. PCA was also recently performed by various authors who investigate genetic diversity in lentil genotypes natives of Pakistan and Turkey respectively^{16,17}. PCs with eigenvalue >1 were considered in defining variability in genotypes which propose variation of genotypes were because of fairly high contribution of few traits rather than little contribution from each trait²⁴. The application of PCA in an assortment of parental lines for breeding purposes has significant importance³⁷. Scattered diagrams revealed that few genotypes were found at distinct positions during 2018 and 2019. Genotypes found in extreme showed high variation to other genotypes. The PC showed that seed yield, biological yield, pod size, hundred seed weight, plant height, and hard seed reflect these genotypes at extreme of scatter diagram. Previous studies showed more than 80% of genetic variation through the first two components^{37,38}.

Hierarchical cluster analysis distributed genotypes based on genetic variation and performance of traits in both years. Genotype 6015 (USA) 5698 (Sialkot, Pak) showed an outlier from all other clusters in 2018 which indicated that this genotype performed differently and both genotypes showed best days to maturity. Genotypes 5664 (Sialkot, Pak) and 6074 (Bahawalnagar, Pak) showed outliers from other clusters in 2019 and placed in cluster 7. Genotypes 5664 and 6074 performed best for the maximum number of quantitative traits as well as seed yield. It was also recorded that Syria's native genotypes grouped into cluster 2 in 2019 while USA and Pakistan's native genotypes grouped together into different clusters. On the other hand genotypes of Syria, USA and Pakistan in 2018 grouped together in C2 clusters. Distribution of 220 lentil genotypes in both years in different clusters noted was probably due to effect of temperature at experimental site. Association among genotypes due to origin separated them from one another based on their genetic behaviour due to genetic variability and presence in the same cluster was due to low variation that could help to trace common ancestor. Considerable genetic variability can play significant role in any lentils breeding program as well as in the improvement of these traits through selection^{24,40}. Large inter-cluster distance between members of any two clusters indicates that genotypes falling in such clusters would be more genetically divergent. Cluster analysis is the best tool to identify the genetic diversity and they classified the 220 lentil genotypes into different clusters based on agro-morphological data as well⁴¹. Generally, genotypes within a group showed little divergence from each other than from genotypes of different groups as has been observed in previous studies^{42,43}.

Structural Equation Model provides a natural vehicle to analyze such complex "multivariate experiments". In our study SEM showed significant level <1 contribution of independent variables like lower pod height, pod size, days to maturity, cooking time, hard seed number of pods, number of seeds per pods toward seed yield as dependent variable. SEM analysis applied to evaluate relationships of seed yield with other dependent variables. To attain desirable yield potential, it is essential to identify major components that have collective impact on total grain yield¹⁶.

Heritability was highest for pod pigmentation followed by leaf pubescence, seedling stem pigmentation, leaf size, hundred seed weight, seed yield, biological yield, pod size, pod shedding while heritability was low in cooking time, ground color of testa. Highest broad-sense heritability was noted for seed yield per plant and number of pods per plant⁴⁴. Most agro-morphological traits had fairly more heritability¹⁸. It is assumed that a greater advance under selection is achieved when characters under selection are highly heritable and stable. Therefore, characters with high heritable value are much more important to a plant breeder than those which are less heritable could enable to develop new combinations for crop improvement programs⁴⁵.

Conclusion

Genotype 6122 originated from Sheikhpura, Pakistan presents best results during both years therefore recommended for farmers field cultivation after conducting necessary trials. As far as biological yield was concerned, genotypes representing Pakistan and Syria exhibited best values for biological yield. Pod pigmentation was absent in 69% genotypes with low Pod dehiscence and shedding that attracts farmers due to harvesting grain losses. Pearson correlation matrix showed significant association among various traits during both years that could contribute to future crop improvement programs to obtain bumper varieties. Highest eigenvalues were shown by F1 2.09 (2018) and F1 2.19 (2019) while studying PCA. Likewise structural equational model displayed significant level < 1 contribution of independent variables (LPH, PS, MD, CT, HS, NP, NSP) toward seed yield as dependant variable. Cluster analysis revealed 04 clusters during 2018 and 07 in 2019 based on quantitative traits revealed geographic distribution independent of origin. Heritability was highest for pod pigmentation, leaf pubescence, seedling stem pigmentation, leaf size, hundred seed weight, seed yield, biological yield, pod size, pod shedding. Twelve genotypes including 5664, 5687, 6084, 6062, 6122, 6058, 6087, 5689, 6042, 6074, Markaz 2009 & Punjab 2009 were selected based on high yield and recommended for future crop improvement programs.

Material And Methods

Plants material

Two hundred and twenty lentil collections from Pakistan, USA and Syria were obtained from National Gene Bank, Plant Genetic Resource Institute, National Agriculture Research Center (NARC), Islamabad, Pakistan (Table 9).

Study site, Research Design and Crop Cultivation

Agro-morphological traits were assessed under field conditions of NARC in Islamabad, Pakistan located at 33.6701° N latitude North and 73.1261° E longitude East, during two consecutive years (2018 and 2019). Monthly temperatures during these years were recorded as is provided in Fig. 8. Experiments were laid out in an augmented design¹⁶. In each year, genotypes were sown on a line of 5 m length, with 10 cm space between plants and 30 cm line to line (row) spacing. Two known cultivars, viz., Markaz 2009 and Punjab 2009, were planted as check (control) after every twenty lines. Recommended cultural practices were followed throughout the crop season.

Table 9
List of lentil genotypes studied during 2018-19.

Obs	Genotypes	Origin	Obs	Genotypes	Origin	Obs	Genotypes	Origin	Obs
1	5472	Sialkot	24	5511	Muzaffargarh	47	5571	Jacobabad	70
2	5474	Gujranwala	25	5512	Muzaffargarh	48	5575	Ghotki	71
3	5475	Gujranwala	26	5517	Muzaffargarh	49	5576	Ghotki	72
4	5476	Gujranwala	27	5518	Muzaffargarh	50	5580	Muzaffargarh	73
5	5477	Gujranwala	28	5527	Sialkot	51	5581	Multan	74
6	5478	Sheikhupura	29	5529	Kasur	52	5583	Muzaffargarh	75
7	5479	Lahore	30	5530	Muzaffargarh	53	5584	Muzaffargarh	76
8	5480	Kasur	31	5531	Muzaffargarh	54	5590	Muzaffargarh	77
9	5481	Kasur	32	5532	Jhang	55	5593	Nawabshah	78
10	5482	Lahore	33	5535	Jhang	56	5595	Kharan	79
11	5483	Sheikhupura	34	5537	Gujrat	57	5598	Kharan	80
12	5484	Sheikhupura	35	5538	Thatta	58	5600	Punjgur	81
13	5485	Kasur	36	5549	Badin	59	5610	Khuzdar	82
14	5486	Kasur	37	5550	Hyderabad	60	5621	Faisalabad	83
15	5488	Okara	38	5553	Hyderabad	61	5622	Faisalabad	84
16	5489	Okara	39	5555	Hyderabad	62	5623	Faisalabad	85
17	5491	Sahiwal	40	5556	Hyderabad	63	5624	Rawalpindi	86
18	5493	Sahiwal	41	5561	Sanghar	64	5625	Muzaffargarh	87
19	5494	Okara	42	5562	Sanghar	65	5626	Muzaffargarh	88
20	5500	Khanewal	43	5563	Sanghar	66	5628	Rajanpur	89
21	5501	Khanewal	44	5564	Sanghar	67	5630	Rajanpur	90
22	5506	Muzaffargarh	45	5565	Hyderabad	68	5634	Layyah	91
23	5510	Muzaffargarh	46	5570	Larkana	69	5635	Larkana	92
116	5696	Narowal	139	5751	Jhelum	162	6015	USA	185
117	5698	Sialkot	140	5753	Rawalpindi	163	6017	USA	186
118	5700	Sialkot	141	5772	Panjgur	164	6037	Syria	187
119	5712	Narowal	142	5773	Kharan	165	6038	Syria	188
120	5716	Narowal	143	5856	USA	166	6041	Syria	189
121	5717	Gujranwala	144	5861	USA	167	6042	Syria	190
122	5723	Layyah	145	5979	USA	168	6043	Syria	191
123	5724	Layyah	146	5981	USA	169	6045	Syria	192
124	5726	Layyah	147	5982	USA	170	6046	Syria	193
125	5727	Layyah	148	5988	USA	171	6047	Syria	194
126	5729	Layyah	149	5993	USA	172	5856	USA	195
127	5730	Layyah	150	5995	USA	173	6052	Syria	196
128	5737	Chakwal	151	5996	USA	174	6054	Syria	197
129	5739	Muzaffargarh	152	5999	USA	175	6058	Syria	198
130	5741	Bahawalpur	153	6002	USA	176	6060	Syria	199
131	5742	Bahawalpur	154	6003	USA	177	6062	Syria	200
132	5744	Faisalabad	155	6005	USA	178	6064	Syria	201
133	5745	Faisalabad	156	6008	USA	179	6066	Bahawalpur	202

Obs	Genotypes	Origin	Obs	Genotypes	Origin	Obs	Genotypes	Origin	Obs
134	5746	Lahore	157	6010	USA	180	6067	Bahawalpur	203
135	5747	Kasur	158	6011	USA	181	6068	Bahawalpur	204
136	5748	Gujranwala	159	6012	USA	182	6069	Bahawalpur	205
137	5749	Gujranwala	160	6013	USA	183	6073	Bahawalnagar	206
138	5750	Jhelum	161	6014	USA	184	6074	Bahawalnagar	207

Data Collection

Two categories of data, quantitative and qualitative were recorded. For quantitative traits, at late vegetative stage ten healthy plants were selected randomly for recording plant height (PH), number of pods per peduncle (NP), lower pod height (LPH), pod size (PS), number of seeds per pod (NSP), and days to maturity (DM) were recorded on foliage, reproductive and at maturity stages. Days to maturity were counted as from sowing day to turning of pods into golden brown of pods (90%). After harvesting biological yield (BY), seed yield (SY), hundred seed weight (HSW), cooking time (CT) [time measured in minutes for cooking unsoaked seed to softness in boiling distilled water at atmospheric pressure] and hard seeds (HS) after boiling 100 seeds were counted.

For qualitative traits, investigations were based on direct observations with categories defined for each trait as mentioned in Table 3. At a late vegetative stage, seedling stem pigmentation, leaflet size and leaf pubescence were observed. At an early reproductive stage, flower ground color was observed. Flower ground color was rated from 1 to 6 according to color shade. 1 indicated white color, 2; white with blue lines, 3; blue, 4; violet, 5; pink, and 6; other. At late reproductive stage, pod pigmentation was observed. At maturity stage, pod dehiscence and pod shedding were surveyed. Pod dehiscence rated from 0 to 7 dehiscence rate. Pod shedding is also rated from 0 to 7. Tendril length was also observed during late reproductive stage. After harvesting, color of pattern on testa, cotyledon color and ground color of test were observed. Color of pattern on testa rated 0 to 4 according to color of pattern. Cotyledon color rated 1 to 3 according to color.

Statistical Analysis

"XLstat 2019" was used to analyze quantitative and qualitative traits data, by which frequency distribution of qualitative traits, Pearson correlation, and PCA analysis were performed. RStudio2019 was explored for hierarchical cluster analysis while "LISREL 10" was employed for regression analysis and Structural Equational Model. Heritability of qualitative and quantitative traits was determined by consulting an online tool "PBSTAT".

Declarations

Competing interest:

No

References

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Figures

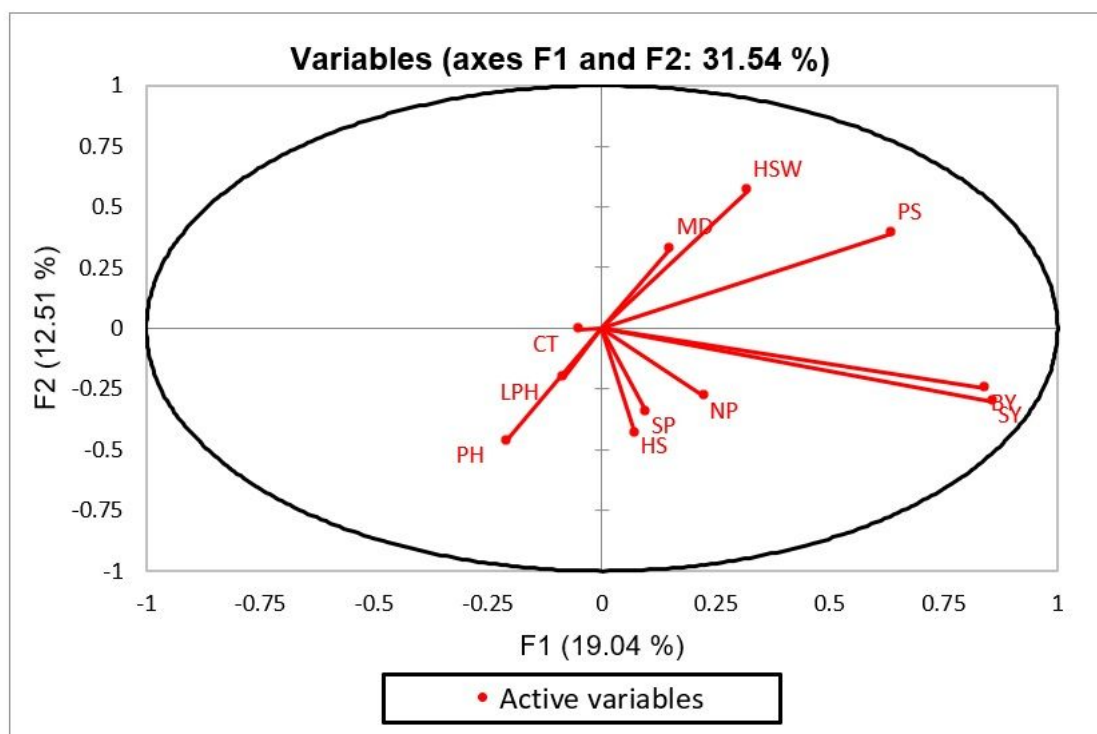


Figure 1

Principal component analyses for quantitative traits of lentil grown during 2018

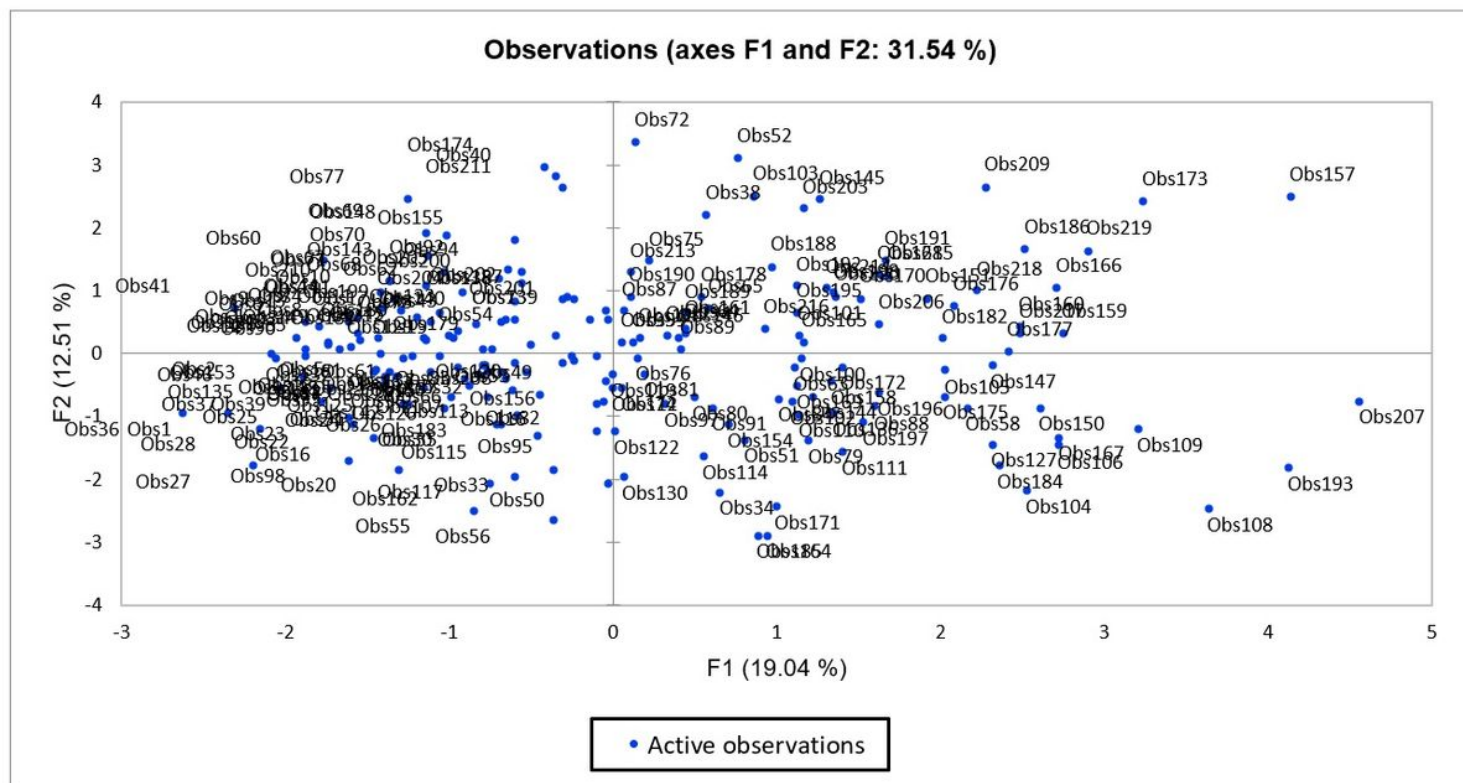


Figure 2

scattered plot of PCA for lentil genotypes grown in 2018

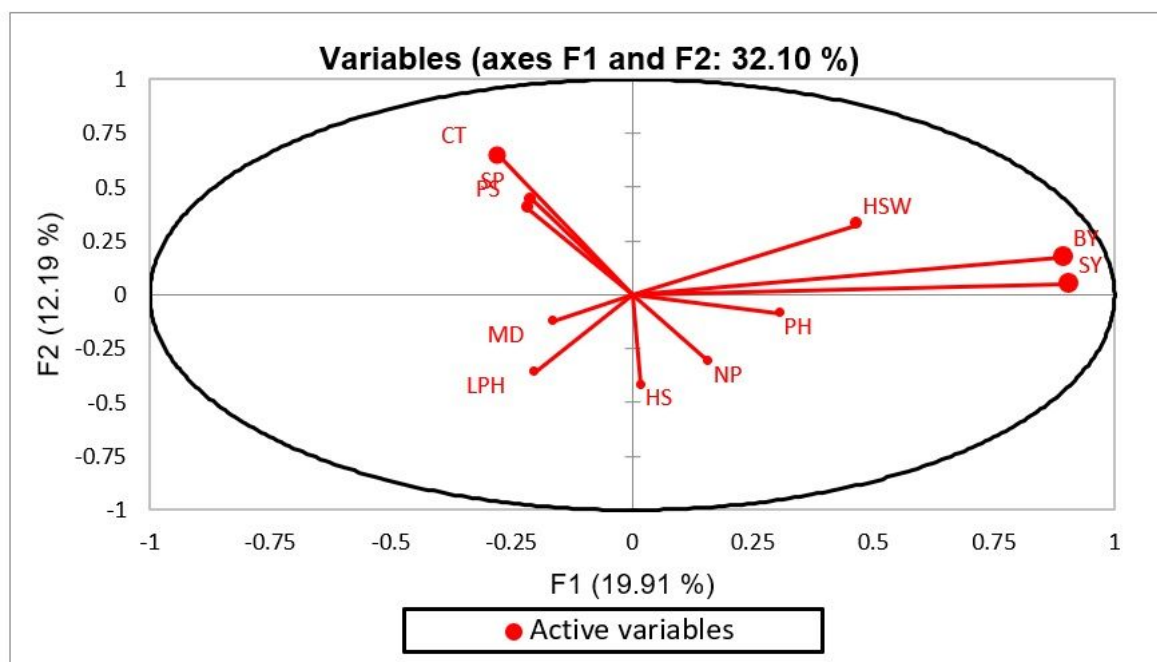


Figure 3

Principal component analysis for quantitative traits of lentil grown in 2019

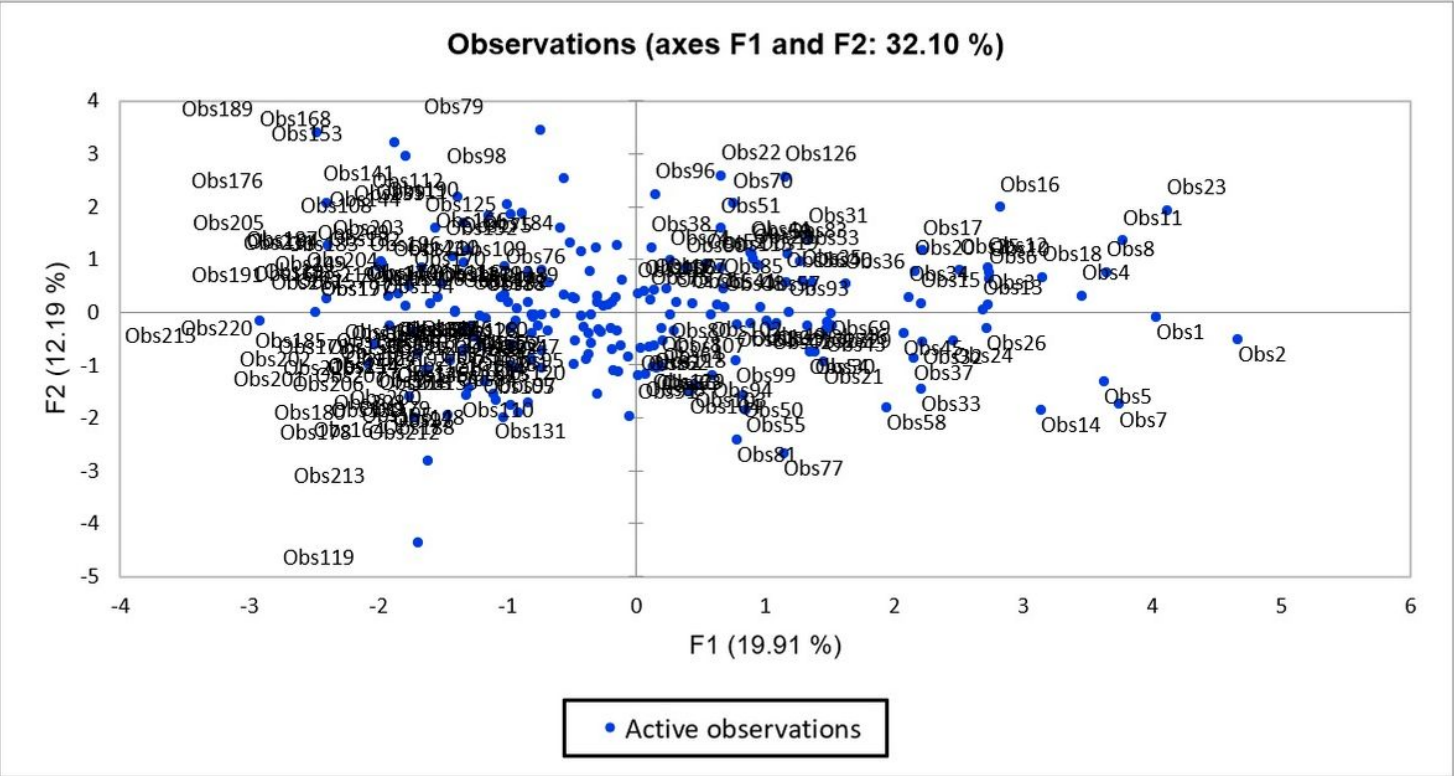


Figure 4

Scattered plot of PCA for lentil genotypes grown in 2019

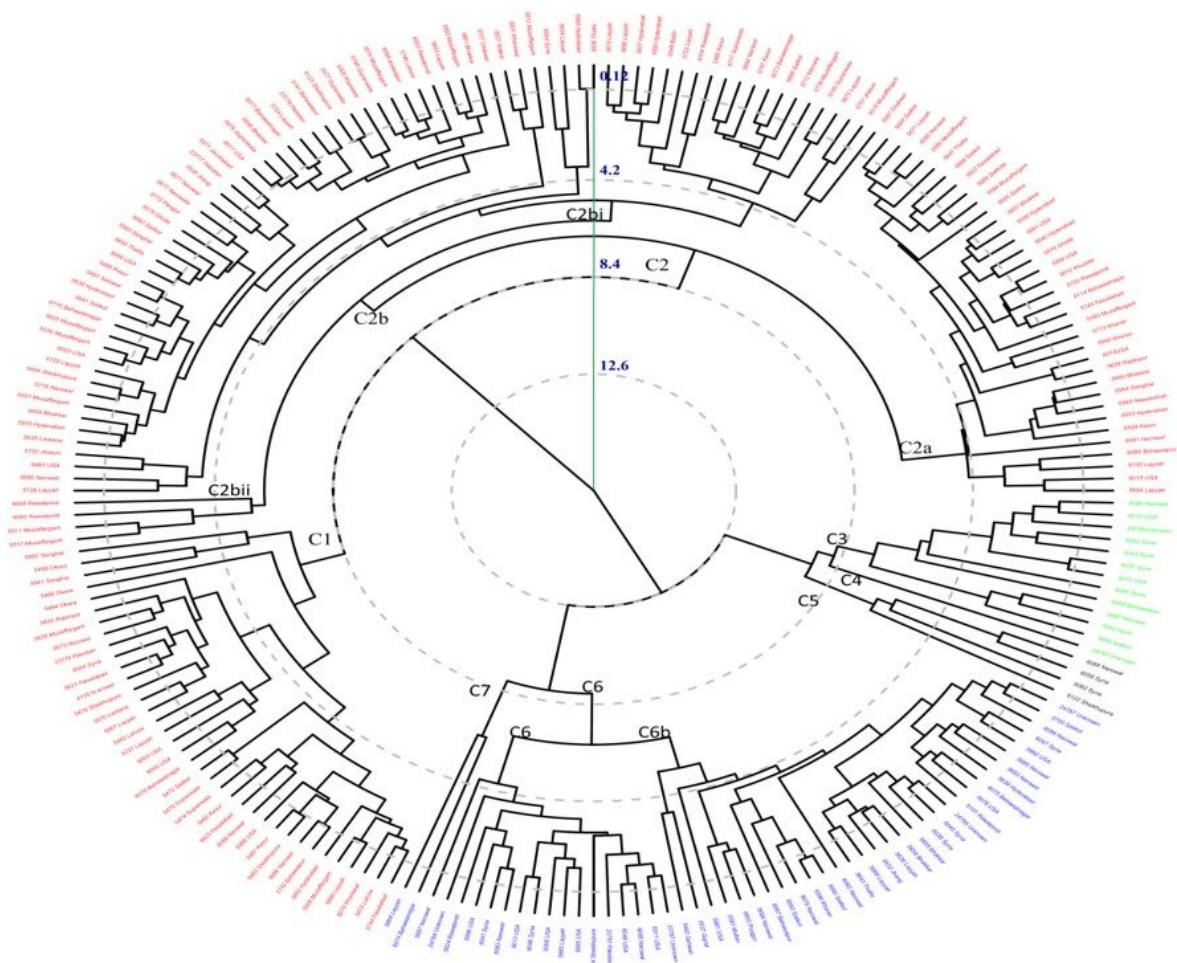
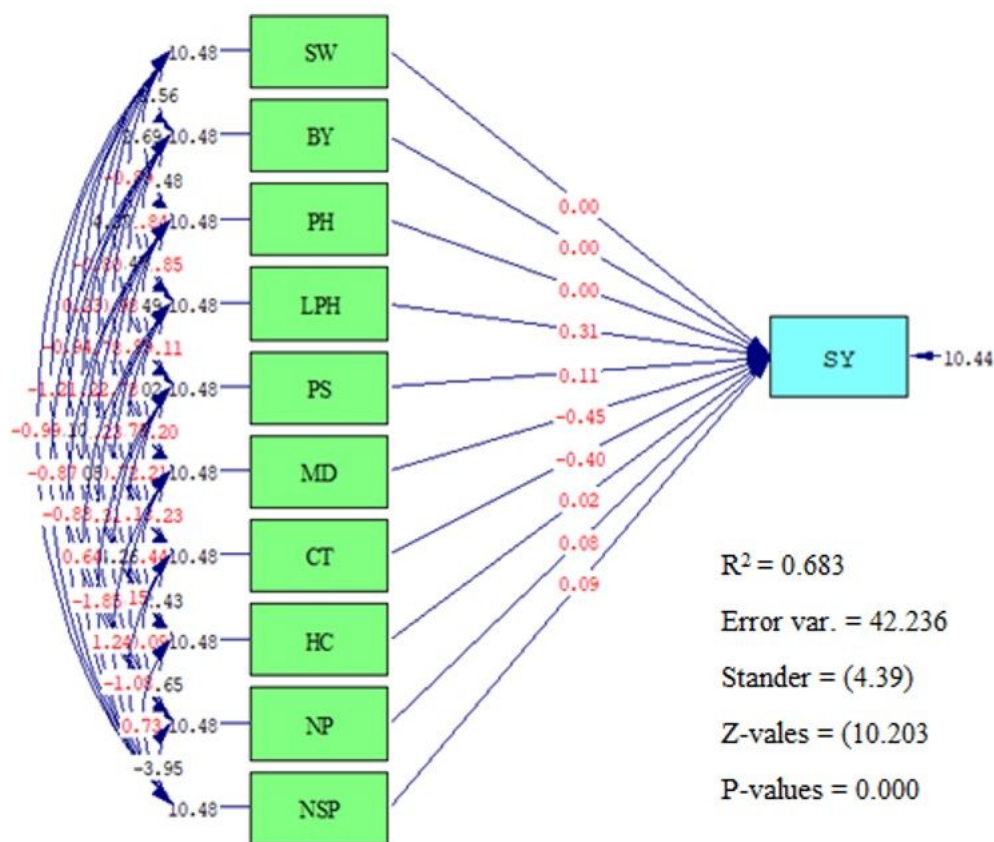


Figure 6

Hierarchical dendrogram showing genetic diversity of lentil genotypes constructed by agro-morphologic data (2019)



Chi-square= 0.00, df=0, P-value=1.00, RMSEA =0.00

Figure 7

Structural Equations of model for seed yield (SY)

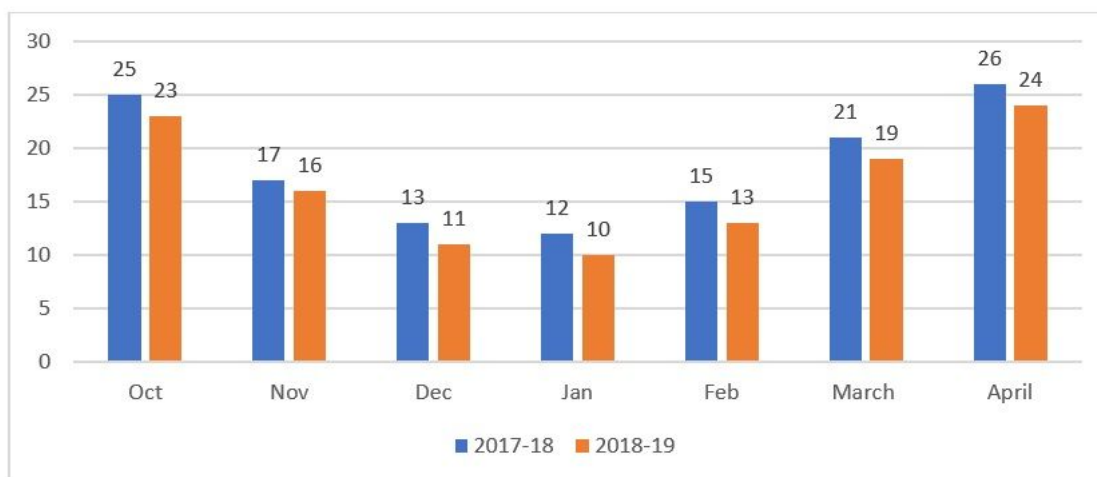


Figure 8

Average temperature during cool-season, 2018 and 2019 in Islamabad, Pakistan