GENETIC VARIABILITY AND HERITABILITY ESTIMATES OF AGRONOMIC TRAITS IN LENTIL (*Lens culinaris* Medik.)

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ABSTRACT

Assessing genetic variability and heritability of the targeted traits is among the crucial first steps toward lentil crop breeding. With the aim of estimating genetic variability and heritability of the crop traits and screening the most promising genotypes, a total of 12 lentil genotypes (11 lines from ICARDA, Syria, and one local cultivar) were evaluated at three locations of the Sulaimani region, northern Iraq during 2018-2019 season. Results showed a significant (P≤0.05) genetic variation among the lentils for most of the agro-morphological traits. In pairwise comparisons, ICARDA lines Flip 93-36L and Flip 2009-70L showed the best performance for most of the evaluated traits and were identified as the most promising for local cultivation and breeding goals. The very significant positive and negative correlations were between seed yield with biomass (r=0.89) and number of days to emergence (r=-0.89). The highest to lowest broad-sense heritability (H^2) values were recorded for 100 seed weight (0.74), number of days to flowering (0.67), first pod height (0.55), biomass (0.47), harvest index (0.37), number of days to maturity (0.36) and 100 seed yield (0.36). Principal components analysis (PCA) supported by cluster analysis was used to analyze the variability among the genotypes based on agro-morphological traits. The first three principal components explained 84.16% of the variability among the genotypes. In addition to the identification of potential lentil genotypes for cultivation uses and lentil breeding in northern Iraq, our variability and heritability data would contribute to the body of lentil breeding knowledge.

Keywords: lentil, genetic variability, broad sense heritability, agro-morphological traits, principal component analysis.

INTRODUCTION

Cultivated lentil (*Lens culinaris* Medik.) is a self-pollinated legume crop with 14 chromosomes (2n=2x=14) and is considered as the most ancient cultivated crop among the legumes (Cokkizgin and Munqez, 2013). Lentils are nutritionally invaluable because they provide a significant level of beneficial nutrients such as proteins, fibres and micronutrients (Karak et al., 2012). According to FAO statistics 2014, the biggest proportion (61%) of lentil production belongs to Asian countries. Concerning the exact origin of this crop, archaeological, botanical and genetic studies emphasize on a core area within the Fertile Crescent comprising southeast of Turkey and North of Syria, near the springs of the Tigris and Euphrates rivers where is supposed to be the real cradle of agriculture (Lev-Yadun et al., 2000; Sonnante et al., 2009).

Study of genetic variability of crops germplasm and estimating the heritability of the traits are among first crucial steps toward breeding crops for desired agronomic traits. Agro-morphological traits have widely been used to investigate genetic diversity among various lentil germplasm including wild accessions (Singh et al., 2020), ICARDA germplasm (Pouresmael et al., 2018), local accessions (Abbasov et al., 2014), and cultivars (Mohammed et al., 2019). For example, in an investigation on the variability of 35 lentil genotypes at agro-morphological levels, Mohammed et al. (2019) identified potential genotypes for dry environment cultivation. In another study on ICARDA germplasm, a significant variations was

reported among the investigated lentil germplasm (Pouresmael et al., 2018).

Broad sense heritability (H^2) is defined as the proportion of phenotypic variance attributed to total genotypic variance including dominance, additive, and epistatic variance (Schmidt et al., 2019). In more simple words, H^2 is defined as a part of phenotypic differences between genotype that can potentially be inherited to the next generations. In breeding programs H^2 is used to predict the response to selection and, hence gives breeders the ability to predict and decide about the success of breeding programs (Piepho and Möhring, 2007; Russu et al., 2019). Broad sense heritability has been estimated for different characteristics of lentil plants including agro-morphological traits (Laskar and Khan, 2017; Singh et al., 2018; Kumar et al., 2018; Debbarma et al., 2018). In a study on 96 lentil genotypes, the H^2 estimation for different agronomic traits ranged between 0.58-0.95 percent (Kumar et al., 2018). In another study a wide H^2 range for different traits from 30.1% for days to maturity to 88.5% for number of fruiting branches have been observed (Singh et al., 2018).

Although Iraq generally and particularly its northern part fall within the main lentil domestication area which harbour a considerable amount of cultivated lentil germplasm, very little research has been done on genetic diversity and heritability of different characteristics including agronomic traits in lentil. In a study on nine ICARDA and local lentil genotypes using agro-morphological traits and RAPD markers Abdulrazzak and Omer (2017) investigated genetic variation and found a high variation among lentils with a considerable part of the variation being between Local and ICARDA populations. Moreover, screening prominent genotypes for particular regions based on environmental conditions would be crucial and helpful for enhancing lentil yield among farmers. Therefore, the main objectives of the present study were to investigate 12 lentil genotypes which are commonly grown in Sulaimani region, northern Iraq, firstly, to assess the phenotypic/genotypic variation and estimate broad-sense heritability of agronomic traits and secondly, to screen and identify prominent genotype(s) in terms of yield performance for cultivation uses across variable environmental condition in the region.

MATERIAL AND METHODS

Plant Materials

The experimental plant material comprised twelve lentil genotypes, including 11 lines introduced from International Center for Agriculture Research in the Dry Areas, ICARDA, Syria and one local cultivar of Sulaimani region (Table 1). The seeds were provided from the Agriculture Research Center in Bakrajo-Sulaimani. These genotypes are the most common lentil genotypes, introduced from ICARDA and are currently cultivating in this region.

Table 1. List of the lentil lines originated from ICARDA (Syria),
with the exception of Sulaimani-Local which is a local cultivar

Genotypes	Origin	Type	Code
Flip 93-36L	ICARDA	line	G1
Flip 04-60L	ICARDA	line	G2
Flip 04-64L	ICARDA	line	G3
Flip 05-51L	ICARDA	line	G4
Flip 2008-7L	ICARDA	line	G5
Flip 2009-24L	ICARDA	line	G6
Flip 2009-52L	ICARDA	line	G7
Flip 2009-54L	ICARDA	line	G8
Flip 2009-64L	ICARDA	line	G9
Flip 2009-70L	ICARDA	line	G10
L10728	ICARDA	line	G11
Sulaimani-Local	Sulaimani	cultivar	G12

Field Experiment

A field experiment was carried out at three locations in Sulaimani region including Bakrajo (location 1) 35°32'42.67"N, 45°21'5.66"E and 738 m, Chamchamal (location 2) 35°31'55.28"N, 44°48'50.66"E and 731 m and Girdjan (location 3) 36°12'33.32"N, 44°47'25.93"E and 533 m (Google Earth Pro, 2020). All three locations are research stations belonging to Agriculture Research Center of Sulaimani. Soil properties for all the three locations are listed in Table 2. The seeds were sown in autumn 2018 at different sowing times on 26th November, 19th December and 4th December of 2018, for all three locations, respectively.

A factorial experiment was run in CRBD design with three replicates. Three environmental conditions were assumed as the levels of first factor and the twelve lentil genotypes were assumed as the levels of second factor. The seed rate of 80 kg/ha was applied for all the genotypes at all three locations. Seeds were planted on lines in plots of 3.2 m^2, each plot comprised of 4 lines, each line with 4 m length and 20 cm distance between the lines. The genotypes were grown in fallow land with no fertilization for all three locations under rainfed condition. Mechanical weed control was applied to the experiment.

Table 2. Some chemical and physical properties of the soil samples in the three locations

Properties	Bakrajo (L$_1$)	Chamchamal (L$_2$)	Girdjan (L$_3$)
Nitrogen%	0.13	0.21	0.19
Phosphorus (ppm)	10.6	12.02	11.708
K$^+$ Meq/L	0.069	0.138	0.071
Organic Matter %	1.75	1.86	1.67
EC ds.m^{-25C}	0.128	0.17	0.14
pH	7.54	8.03	7.95
Cl$^-$ Meq/L	0.6	0.6	0.4
CaCO$_3$%	18	15	29

Collecting agro-Morphological data

The agro-morphological data were measured following the descriptors proposed by the International Plant Genetic Resources Institute (IPGRI). Data were recorded from the whole plot for seed yield, biomass, and harvest index, while for other traits the average value from ten plants in each plot was recorded. The measured traits include: Seed yield (SY) in kg/hectare as a rate of yield per hectare by tons, counted by converting the yield of plot area into a hectare, Days to emergence (DE), counted as the number of days spent from sowing till the emergence of the first seedling, Days to 50% flowering (DF), Days to 50% maturity (DM), First pod height (FPH), 100 seed weight (SW), Biomass (B), was the average weight of the whole plants for each plot converted to ton/ha, and Harvest index (HI), counted as the ratio of grain yield to biological yield, according to Rehman et al. (2009).

$$\text{Harvest index (HI)} = \frac{\text{Economic yield (seed yield)}}{\text{Biological yield}} \times 100$$

Estimating Broad sense heritability

Broad sense heritability (H^2) was measured using a combined variance component procedure according to Comstock and Moll (1963) using a mixed model with genotype × locations interactions considering location and blocks as random genotypes as a fixed variable. After extracting variance components i.e. genotypic ($\sigma^2 g$), genotype × location interaction ($\sigma^2 gl$) and error ($\sigma^2 e$) variances from combined ANOVA table, H^2 was measured using the following formula.

1. $$\sigma^2 g = \frac{MSG - \sigma^2 e - \sigma^2 gl}{RL}$$

Where MSG is the mean square of genotype variable in the ANOVA table, R is the number of replications and L is the number of locations.

2. $\sigma^2 gl = \frac{MSGL - \sigma^2 e}{R}$

Where MSGL is the mean square of genotype $\times$ location interaction and R is the number of replications.

3. $\sigma^2 p = \sigma^2 g + \sigma^2 gl + \sigma^2 e$

4. $H^2 = \frac{\sigma^2 g}{\sigma^2 p} = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 gl + \sigma^2 e}$

Data Analysis
Data collected for all the three locations were subjected to the combined analysis of variance (ANOVA) based on factorial CRBD experimental design. Means were compared by using Duncan's test at the 0.05 probability level. Prior to the ANOVA analysis, Bartlett test was used for the test of variance homogeneity of the data. ANOVA, compare means for both genotypes and locations for agro-morphological traits and Pearson correlation coefficient between traits was performed using XLSTAT version 2016 and SPSS Statistics Version 2019 (SPSS Inc., IBM Company). Hierarchical clustering

based on Euclidean distance coefficients and Principal component analysis (PCA) was analyzed using XLSTAT software.

RESULTS AND DISCUSSION

Analysis of variance
A considerable variation among the genotypes was found for the agro-morphological traits. The results of the analysis of variance revealed a significant ($P \leq 0.05$) genotype variation for agro-morphological traits: seed yield (SY), days to 50% flowering (DF), days to 50% maturity (DM), first pod height (FPH), 100 seed weight (SW), biomass (B), and harvest index (HI). However, the genotype effect was not significant on days to emergence (DE). Also, the effects of location and the interaction of genotype $\times$ location was statistically significant ($P \leq 0.05$) for all of the evaluated traits (Table 3). These data indicate a significant genotypic variation for the studied agro-morphological traits, except DE and phenotypic variation resulted from environmental factors or their interaction with genetic factors.

Table 3. Two-way ANOVA results for different agronomic traits of studied lentil genotypes

	ANOVA (MS±SE)[a]		
Trait	Genotype	Location	Genotype × Location
SY	156065±43816**	7510202.4±41082**	45097± 8471**
DE	14.7±16.52	3512.1±17.5**	16.6±0.48**
DF	353±47.7**	10152.5±46.8**	47.5±1.1**
DM	97.4±34.8*	9654.6±35.3**	34.8±0.6**
FPH	59.2±9**	483.7±13.3**	9±2.2**
SW	2.9±0.27**	4.6±0.4**	0.27±0.01**
B	823499.8±8493**	54474356.6±142163**	156701±41324.3**
HI	0.015±0.002**	0.21±0.002**	0.003±0.001*

*, **: significant at 0.05 and 0.01 levels, respectively.
[a] the numbers represent mean square ± standard error.

Pairwise comparisons were carried out between the three locations and between the 12 genotypes using Duncan's comparison of the means. According to the comparisons, significant differences were observed between all three locations (Table 4).
Under conditions of locations 1 and 3 the values for traits SY, B, DE, SW and FPH

were close but different from those of location 2. For example, the means of SY in locations 1 and 3 were 1027.9 and 1186.2, whereas it was 327.9 in location 2. Also, while DE mean was 36.9 in location 2, it was 21.4 and 18.5 in locations 1 and 3, respectively. Soil fertility and dried condition especially during growth and seed filling

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period could be one of the reasons for the highest seed yield obtained in Bakrajo and Girdjan (location 1 and 3) compared to the yield of location 2. Differences in the elevation between Bakrajo and Girdjan (300 m) seems to have no negative impact on the performance of lentil at both locations, being less sensitive to the climate and soil features, as lentil has the ability to grow in wide environmental condition and elevation rate from 200 m up to 3000 m above sea level (Ansari et al., 1990; Cokkizgin and Munqez, 2013). The means of seed yield among the genotypes over the locations ranged from 670.2 to 1084 kg/ha. In pairwise comparisons, genotypes G10 and G1, with means of 1084.4 and 1019.4 kg/ha, showed the highest performance for SY. In addition, G1 and G10 along with G12 produced the highest amount of biomass among all the genotypes (Table 4).

Table 4. Duncan's compare means results for genotype and locations

Genotype[a]/ Location	Traits							
	SY	DE	DF	DM	FPH	SW	B	HI
G1	1019.4[ab*]	23.7[e]	104.1[j]	148.2[g]	11.4[b]	3.1[f]	2537.7[a]	0.417[abc]
G2	670.2[h]	24.7[d]	115.6[de]	154.4[d]	7.8[de]	2.7[i]	1750.1[e]	0.400[c]
G3	906.1[cd]	25.0[cd]	114.6[e]	152.8[e]	9.1[cd]	4.5[a]	2143.5[bc]	0.427[abc]
G4	812.5[efg]	24.9[cd]	111.0[g]	153.4[e]	9.1[cd]	3.4[d]	1876.6[de]	0.427[abc]
G5	670.6[h]	25.6[c]	112.2[f]	155.2[cd]	7.1[ef]	2.9[h]	1700.6[e]	0.404[bc]
G6	886.0[cde]	24.3[de]	111.8[fg]	155.1[cd]	9.5[c]	3.6[c]	2122.4[bc]	0.424[abc]
G7	787.3[fg]	24.3[de]	105.7[i]	153.0[e]	8.8[cd]	3.5[cd]	2028.2[cd]	0.408[bc]
G8	778.2[fg]	26.4[b]	116.3[d]	155.6[c]	9.1[cd]	2.4[j]	2057.8[bcd]	0.389[c]
G9	971.4[bc]	27.6[a]	118.2[c]	155.1[cd]	11.4[b]	2.9[gh]	2262.6[b]	0.443[ab]
G10	1084.4[a]	26.7[b]	109.0[h]	149.1[f]	5.8[f]	3.0[fg]	2508.1[a]	0.448[a]
G11	846.2[def]	26.9[ab]	120.7[b]	157.3[b]	13.3[a]	3.8[b]	2172.1[bc]	0.411[abc]
G12	736.2[gh]	27.0[ab]	126.3[a]	160.4[a]	14.8[a]	3.3[e]	2654.1[a]	0.292[d]
Location 1	1027.9[b]	21.4[b]	130.4[a]	165.2[a]	13.6[a]	3.2[b]	3160.2[a]	0.33[c]
Location 2	327.9[c]	36.9[a]	114.1[b]	161.9[b]	6.3[c]	3.7[a]	780.87[c]	0.42[b]
Location 3	1186.2[a]	18.5[c]	96.9[c]	135.3[c]	9.3[b]	2.9[c]	2512.4[b]	0.48[a]

* means with the same lowercase have no significant difference at p=0.05 level.

Both G1 and G10 with the highest seed yield, biomass and harvest index showed to be the most productive out of all the genotypes. High variations in the seed yield of the lentil genotypes is in accordance with the results other researchers (Vanda et al., 2013; Mekonnen et al., 2014; Sharma et al., 2014). Biomass is important characteristic and its improvement especially at the early growth stage would contribute further in assimilation of nitrogen to have reflecting the seed productivity (Whitehead et al., 1980).

From the viewpoint of earliness in maturity and ripening which would be crucial in arid and semi-arid climates, G1 showed the best performance, requiring fewer days to emergence (DE) flowering (DF) and maturity (DM). Highly significant differences among 16 lentil genotypes for the studied agro-morphological traits in Hallabja province have been identified by Ali et al. (2017), including the genotypes sourced from ICARDA with local cultivars. The analysis of variance in the current study revealed highly significant differences among lentil genotypes at all three locations, indicating a high degree of genetic variation, having great potential in future breeding programs through selection. In other studies, agro-morphological traits have been used to study variation among lentil genotypes and to screen potential genotypes for cultivation and breeding as

well (Abdulrazzak and Omer, 2017; Debbarma et al., 2018). In a study on 10 lentil genotypes, three genotypes were identified as suitable for seed production based on genetic factors such as earlier development and seed yield quantification in individual plants (Debbarma et al., 2018). In another investigation on ICARDA germplasm, quantitative traits grain yield, seed weight per plant and pod weight per plant indicated the most coefficient of variation among a set of quantitative traits. In addition, the genotypes with the highest performance for 100 seed weight and grain yield were identified (Pouresmael et al., 2018). In an investigation on lentil genotypes in dry environments Mohammed et al. (2019) reported very less time needed for the development of the genotypes than what found in the present study which could be because of the critical environmental differences between the regions under which the two studies had been carried out, or the genetic construction could be the reason. Overall, among all genotypes investigated in this study, G1 showed to be best for the evaluated traits except for FPH and SW which showed lower ranks that could be attributed to negative correlations between the traits (Table 4). Therefore, G1 followed by G10 are suggested as suitable genotypes to be introduced to farmers in the region for direct cultivation as well as to be employed in future lentil breeding programs of high yield potential and more resistant to the local uncertain environmental condition and maybe for other rainfed regions with similar environmental conditions.

Correlations between the traits

Understanding relationships among the characters is required for improving a target character via indirect selection of characters more heritable and easier to select (Dugassa et al., 2015). According to Pearson's correlation coefficients between the traits, there was a significant high negative correlation ($P \leq 0.01$, r=-0.89) between SY and DE (Table 5). Whereas, there were significant negative correlations between SY

with DF (r=-0.21, $P \leq 0.05$) and DM (r=-0.53, $P \leq 0.01$). The correlation between SY and SW was significantly negative. Although, biomass (B) showed strong positive correlations with either SY or FPH, it was negatively correlated to either DE, DM or SW. Furthermore, there was a significant positive correlation between SW and DE and DM. However, harvest index (H) was negatively correlated to DF, DM, FPH and B. A negative correlation between SY and prolonged development time was reported in other studies. Depar et al. (2016), concluded that prolonged flowering and maturity of lentil had an adverse effect on yield which is consistent with our study concerning the negative correlation between seed yield with DF and DM traits.

Growing lentil under rainfed condition of the current investigation making clear reduction in the yield by shrinking the flowering and seed filling period. Yield reduction of lentil has been identified by delaying flowering in an investigation by (Yuan et al., 2017). In agreement with our results, other investigations reported negative correlations between seed yield and number of days to maturity (Debbarma et al., 2018) and days to flowering (Abo-Hegazy et al., 2012). Overall, the correlation data presented here suggests giving emphasis on earliness (earlier DE and DM) and biomass in lentil breeding for improved yield for an environmental condition like what given here. A variation of three weeks for days to flowering among the studied genotypes is considered as an effective mechanism for drought escape and seed setting before the onset of terminal drought in grain legumes (Zhang et al., 2000; Shrestha et al., 2006), because the effect of water deficit during the reproductive and grain filling stages is more critical on the yield loss (Farooq et al., 2009; Pushpavalli et al., 2015), especially for the rainfed agriculture condition of Sulaimani Region. The major contributed traits to yield could be emphasized during the development of the improved genotypes of lentil for rainfed conditions (Bhartiya et al., 2015).

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Table 5. Pearson's coefficients of correlation (r) and coefficients of determination (R^2)
between 8 agro-morphological traits

Trait[a]	SY[b]	DE	DF	DM	FPH	SW	B	HI
SY		-0.89**	-0.21*	-0.53**	0.46**	-0.35*	0.86**	0.17
DE	0.79		0.19*	0.51**	-0.45**	0.41**	-0.82**	-0.03
DF	0.04	0.04		0.89**	0.50**	0.16	0.21*	-0.78**
DM	0.28	0.26	0.79		0.21*	0.31*	-0.15	-0.68**
FPH	0.21	0.26	0.25	0.04		-0.07	0.72**	-0.50**
SW	0.12	0.17	0.03	0.16	0.00		-0.32**	-0.03
B	0.74	0.67	0.04	0.02	0.52	0.10		-0.33**
HI	0.03	0.00	0.61	0.46	0.25	0.00	0.11	

[a] the numbers above the diagonal of table indicate the correlation coefficients between different traits and those below the diagonal indicate the coefficients of determination (R^2).

**, * correlations are significant at 0.01 and 0.05 levels respectively (2-tailed).

Broad sense heritability (H^2) of the traits

Following the extraction of variance components from the ANOVA table, broad-sense heritability (H^2) was calculated for the evaluated agro-morphological traits (Table 6). The highest value of H^2 (0.74) was estimated for 100 seed weight (SW).

The H^2 value of DF and FPH was 0.67 and 0.55, respectively, showing that these three traits are highly under the control of genetic factors and the genetic response and breeding progress would be more predictable for these traits than other traits with lower H^2 values.

Table 6. Estimation of variance components and broad sense heritability
for the evaluated traits among the lentil genotypes

Trait	Variance components				H^2
	$\sigma^2 e$	$\sigma^2 gl$	$\sigma^2 g$	$\sigma^2 p$	
SY	08471.23	12208.50	11388.55	32068.28	0.36
DE	0.48	5.38	-0.27	5.60	0.00
DF	1.13	15.45	33.81	50.39	0.67
DM	0.61	11.41	6.88	18.90	0.36
FPH	2.16	2.28	05.35	9.78	0.55
SW	0.01	0.09	0.29	0.39	0.74
B	41324.33	38458.89	69497.17	149280.39	0.47
HI	0.0015	0.0005	0.0011	0.0031	0.37

$\sigma^2 e$: error variance (environment effect); $\sigma^2 gl$: genetic × location variance; $\sigma^2 g$: genetic variance.
$\sigma^2 p$: phenotypic variance; H^2: broad sense heritability.
SY - Seed yield; DE - Days to emergence; DF - Days to 50% flowering; DM - Days to 50% maturity;
FPH - First pod height (cm); SW - 100 seed weight; B - Biomass (kg/ha); HI - Harvest index.

Supporting these results, Debbarma et al. (2018) reported a high H^2 and genetic advance for a set of agro-morphological traits including days to first flowering, days to maturity, 1000 seed weight and seed yield per either plant or plot. In another study in Diyarbakir, Turkey where is geographically and environmentally close to our region, Bicer and Sakar (2010) estimated H^2 values for days to flowering, days to maturity and seed weight as 71%, 80%, 98%, respectively. These are partly consistency with our results

for SW, DF and DM (Table 5). In a one year and one location basis, Dugassa et al. (2015) estimated the heritability values for biomass yield and days to emergence as >60% while for days to maturity, days to flowering, and harvest index as between 40% and 60% which are close to our results while they neither considered experiment repetition for years nor locations. The H^2 value for DE was estimated 0.00 which could be due to the non-significant genetic variance of this trait (ANOVA results, Table 3) resulted from high

differences between location 2 with locations 1 and 3 leading to critical effects of both location and location × genotype interaction dominating over pure genotype effects. Whereas, one-way ANOVA for the genotype effect in any of the locations separately, showed a significant genotype effect for DE (data not shown).

Hierarchical clustering and principal component analysis

The Euclidean distance and unweighted pair-group average were followed to estimate dissimilarity between the lentil genotypes.

The dissimilarity values were varied from the lowest value of 0.99, between G2 and G5, to the highest of 7.038, between G1 and G12 (Table 7). Cluster analysis clustered the genotypes into 3 main groups (Figure 1). Out of 12, seven genotypes were grouped into the largest cluster including two sub-clusters. The first sub cluster included G3, G6, G4 and G7 and the second one included G2, G5 and G8. Both G1 and G10 gathered in a cluster while G11 and G9 grouped together and with a less similarity grouped with G12, a local genotype from the Sulaimani region.

Table 7. The Euclidian distances used for hierarchical clustering between 12 lentil genotypes

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
G1	0											
G2	4.895	0										
G3	3.961	4.019	0									
G4	3.631	2.076	2.310	0								
G5	5.117	0.990	3.989	2.027	0							
G6	3.266	2.850	1.866	1.258	2.933	0						
G7	3.182	2.576	2.569	1.214	2.512	1.516	0					
G8	4.684	2.088	4.188	2.649	2.062	3.042	3.187	0				
G9	4.488	4.080	3.773	3.289	3.971	3.227	4.030	2.598	0			
G10	3.451	4.962	4.020	3.866	4.800	3.801	3.986	4.207	3.493	0		
G11	5.157	4.106	3.101	3.256	4.059	3.009	3.909	3.223	2.390	5.092	0	
G12	7.038	5.874	6.077	6.029	6.024	5.678	6.217	4.675	5.056	7.378	3.871	0

This clustering results support the results of the pairwise comparisons based on the compare means data (Table 3). For example, G1 and G10 which were identified as the best for seed yield earliness, biomass and harvest index, were clustered together as well. Additionally, nearly such coordination was between the two sets of the data for other groups of the genotypes.
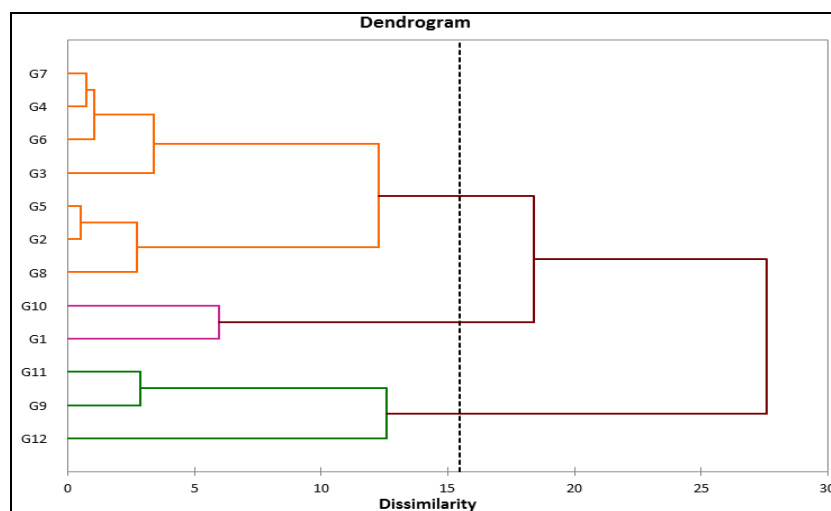

Figure 1. Hierarchical clustering of 12 lentil genotypes based on Euclidean distance using the data from three locations and eight agro-morphological traits

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Transformation of the evaluated traits into principal components (PCs) produced 8 eigenvectors. The first eigenvector expressed the highest eigenvalue 3.56 and only first three Principal Components (PCs) gave eigenvalues more than one (Table 8) and thus were considered in determining the variability of agro-morphological traits among the genotypes (Kaiser, 1960). This suggests that variation among the genotypes was due to a relatively high contribution of fewer traits rather than a small contribution of each trait. Principal component analysis (PCA) reflects the importance of the largest contributor to the total variation at each axis of differentiation and identifies the traits that contribute most to the variation within genotypes (Bhartiya et al., 2015). The first three PCs explained 84.16% of the total variation with PC1, PC2 and PC3 accounting for 44.5%, 25.9% and 14.7% of total variation, respectively (Table 8 and Figure 2).

Table 8. The eigenvalues and related variability for corresponding 8 component characters
explained by the principal components used in the analysis of the variation among 12 lentil genotypes

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigenvalue	3.561	1.996	1.176	0.853	0.282	0.075	0.053	0.003
Variability (%)	44.514	24.947	14.705	10.663	3.530	0.943	0.666	0.032
Cumulative (%)	44.514	69.461	84.166	94.829	98.359	99.303	99.968	100.000

The largest percent of variation was contributed in PC1 by DF and DM (24.3% each), PC2 by B and SY (41.4 and 35.7) and PC3 by DE and SW (28.6 and 58.2), respectively (Table 9). Analysis of PCA was used to study the variability of agro-morphological traits in lentil germplasm in India (Kumar and Solanki, 2014; Bhartiya et al., 2015), Saudi Arabia (Mohammed et al., 2019), Algeria (Gaad et al., 2018), Bangladesh (Ahamed et al., 2014) and wild lentil accessions around the world (Singh et al., 2020). Bhartiya et al. (2015) showed that the first four components of PCA explained 83.3% of the cumulative variance and highly significant positive correlation of PCI was observed with harvest index, number of pods per plant, days to maturity, seed yield per plant and days to 50% flowering. Moreover, Kumar and Solanki (2014) reported the maximum contribution of days to 50% flowering, plant height and days to maturity to total diversity among lentil accessions.

Table 9. Contribution of the lentil agro-morphological traits to the variation explained
by first three principal components (PCs) used to analyze the variation among 12 lentil genotypes

Characteristics	PC1	PC2	PC3
Days to emergence (DE)	10.460	4.773	28.612
Days to 50% flowering (DF)	24.333	0.142	0.725
Days to 50% maturity (DM)	24.301	3.045	0.563
First pod height (cm) (FPH)	14.480	9.942	8.431
100 seed weight (SW)	0.033	4.522	58.184
Biomass (kg/ha) (B)	1.004	41.449	0.257
Seed Yield (kg/ha) (SY)	6.838	35.718	1.806
Harvest index (H)	18.551	0.409	1.423

The PCA biplot of the first two PCs separated the 12 lentil genotypes on the plot (Figure 2). The genotypes were gathered in as similar groups as for cluster analysis and also supported the data of compare means and pairwise analyses. As can be observed on the plot, G1 and G10 that were identified as the most promising for cultivation and future breeding were grouped together.
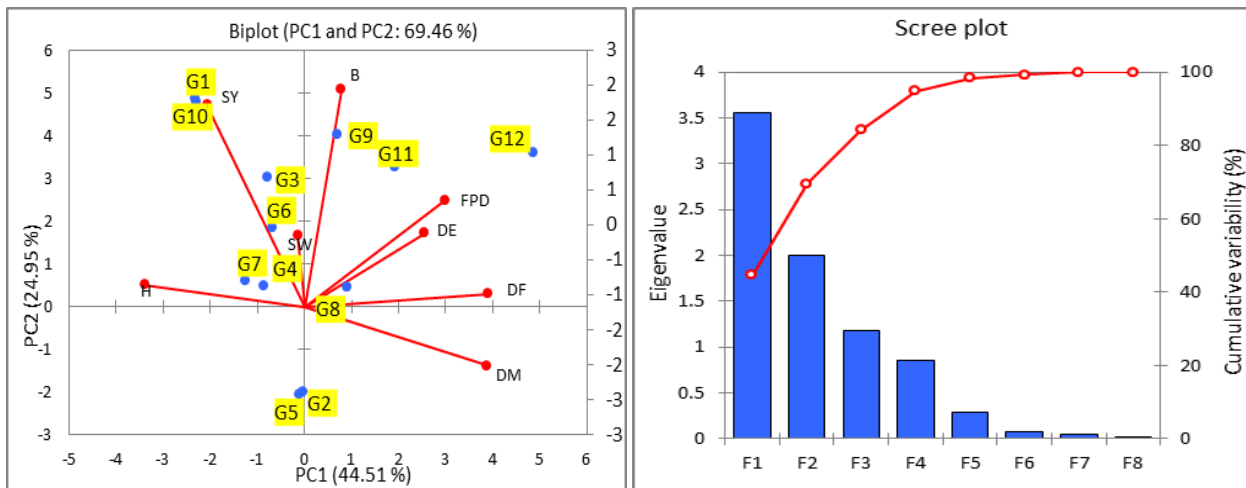
Figure 2. Pricipal component (PCA) biplot (left) showing the distribution of 12 lentil genotypes (blue dots)
based on data from three locations and eight evaluated traits (red dots).
The scree plot (right) showes the variability given by eight principal components (F1- F8).
SY - Seed yield; DE - Days to emergence; DF - Days to 50% flowering; DM - Days to 50% maturity;
FPH - First pod height (cm); SW - 100 seed weight; B - Biomass (kg/ha); HI - Harvest index.

Like the cluster analysis, the local genotype (G12) was positioned close to G9 and G11 while G5 and G2 grouped together and the other genotypes were scattered close together on the up-left quarter of the plot. The local cultivar (G12) with G9 and G11 seem to be sharing the same ancestor, by other mean both G9 and G11 could have better performance to cope with the uncertain abiotic fluctuation as the local does in adaptation. Altogether, the PCA results supported those of hierarchical clustering and both are in accordance with the pairwise analysis of the genotypes differences/similarities for the evaluated agro-morphological traits.

CONCLUSIONS

In this study, genetic variability and broad-sense heritability among 12 lentil genotypes were estimated for eight agro-morphological traits.

The highest level of broad-sense heritability was found for seed weight followed by days to 50% flowering flowed and first pod height.

Among lentil genotypes, two ICARDA lines Flip 93-36L (G1) and Flip 2009-70L (G10) were proven to be the most suitable for cultivation under rainfed condition of Sulaimani region, northern Iraq, however

further field trial is recommended to optimize the best sowing date for the lentil genotypes under rainfed condition of the region. These genotypes showed the best performance for yield and earliness in growth and development and therefore can potentially be valuable sources for genetic analysis and future lentil breeding in this region.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiment: N.S.A, J.G.R and D.J.M. Analyzed the data: N.S.A. and N.M. Contributed materials/analysis tools: N.S.A., N.M. and J.G.R. Wrote the paper: N.S.A. and N.M.

ACKNOWLEDGEMENTS

REFERENCES

Abbasov, M., Babayeva, S., Akparov, Z., Damania, A., Izzatullayeva, V., Aslanova, G., 2014. *Genetic diversity for drought tolerance in lentils from Central Asia and the Caucasus: CACLentil*. Albanian J. Agric. Sci., 13: 1-8.
Abdulrazzak, N., and Omer, D.A., 2017. *Genetic variation in lentil genotypes by morpho-agronomic traits and RAPD-PCR*. J. Anim. Plant Sci., 72: 468-480.

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Abo-Hegazy, S.R.E., Selim, T., El-Emam, E.A.A., 2012. *Correlation and path coefficient analyses of yield and some yield components in lentil.* Egypt J. Plant Breed, 16: 147-159. https://doi.org/10.12816/0003954

Ahamed, K., Akhter, B., Islam, M., Humaun, M., Alam, M., 2014. *Morphological characterization and genetic diversity in lentil (Lens culinaris medikus ssp. Culinaris) germplasm.* Int. J. Agric. Res. Innov. Technol., 4: 70-76. https://doi.org/10.3329/ijarit.v4i1.21095

Ali, F.N.A., and Layeeq, T., 2017. *Analysis of genetic diversity in lentil (Lens culinaris medik.) germplasm using agro-morphological and DNA based markersle.* MSc thesis, Sulaimani Polytechnic University.

Ansari, S.A., Afridi, S.M.M.R.K., Khan, N.A., 1990. *Response of field-grown lentil to pre-sowing seed enrichment with pyridoxine.* F. Crop Res., 23: 45-53. https://doi.org/10.1016/0378-4290(90)90096-T

Bhartiya, A., Aditya, J.P., Singh, S., 2015. *Assessment of variability for agro-morphological traits in elite lentil (Lens culinaris) lines using multivariate analysis.* Indian J. Agric. Res., 49: 539-543. https://doi.org/10.18805/ijare.v49i6.6682

Bicer, B.T., and Sakar, D., 2010. *Heritability of yield and its components in lentil (Lens culinaris medik.).* Bulg. J. Agric. Sci., 16: 30-35.

Cokkizgin, A., and Munqez, J.Y.S., 2013. *Lentil: origin, cultivation techniques, utilization and advances in transformation.* Agric. Sci., 1: 55-62. https://doi.org/10.12735/as.v1i1p55

Comstock, R.K., and Moll, R., 1963. *Genotype-environment interactions.* In: W.D. Hanson and H.F. Robinson (eds.), Statistical Genetics and Plant Breeding. National Academy of Sciences, National Research Council Publication, 982: 164-169.

Debbarma, M., Laloo, B., Mandal, J., Chakraborti, P., 2018. *Genetic variability in yield attributes of lentil genotypes under new alluvial zone.* Curr. J. Appl. Sci. Technol., 30(5): 1-6.

Depar, M.S., Soomro, N.A., Chandio, A.S., Samoon, H.A., Meghwar, B.L., Pathan, A.K., Kalroo, M.W., 2016. *Evaluation of morpho-yield traits and their correlation with seed yield in lentil (Lens culinaris) Genotypes.* Sci. Int., 28: 2675-2678.

Dugassa, A., Legesse, H., Geleta, N., 2015. *Genetic variability, yield and yield associations of lentil (Lens culinaris Medic.) genotypes grown at Gitilo Najo, Western Ethiopia.* Sci. Technol. Arts. Res. J., 3: 10. https://doi.org/10.4314/star.v3i4.2

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., 2009. *Plant drought stress: Effects, mechanisms and management.* In: Sustainable Agriculture, Springer Netherlands: 153-188.

Gaad, D., Laouar, M., Gaboun, F., Abdelguerfi, A., 2018. *Collection and agro morphological characterization of algerian accessions of lentil (Lens culinaris).* Biodiversitas, 19: 183-193. https://doi.org/10.13057/biodiv/d190125

Kaiser, H.F., 1960. *The application of electronic computers to factor analysis.* Educ. Psychol. Meas., 20: 141-151. https://doi.org/10.1177/001316446002000116

Karak, T., Erdem, H., Baloch, F.S., Toklu, F., Eker, S., Kilian, B., Ozkan, H., 2012. *Diversity of macro- and micronutrients in the seeds of lentil landraces.* Sci. World J.: 1-9. https://doi.org/10.1100/2012/710412

Kumar, J., Solanki, R., 2014. *Evaluation of lentil germplasm for agro-morphological traits.* J. Food Legum., 27: 302-306.

Kumar, J., Gupta, S., Gupta, D.S., Singh, N.P., 2018. *Identification of QTLs for agronomic traits using association mapping in lentil.* Euphytica, 214: 75. https://doi.org/10.1007/s10681-018-2155-x

Laskar, R.A., and Khan, S., 2017. *Assessment on induced genetic variability and divergence in the mutagenized lentil populations of microsperma and macrosperma cultivars developed using physical and chemical mutagenesis.* PLoS One, 12: 1-18. https://doi.org/10.1371/journal.pone.0184598

Lev-Yadun, S., Gopher, A., Abbo, S., 2000. *The cradle of agriculture.* Science, 288: 1602-1603.

Mekonnen, F., Mekbib, F., Kumar, S., Ahmed, S., Sharma, T.R., 2014. *Agromorphological traits variability of the ethiopian lentil and exotic genotypes.* Adv. Agric.: 1-15. https://doi.org/10.1155/2014/870864

Mohammed, N.A., Refay, Y.A., Migdadi, H.M., Al-Somain, B.H., Muharram, A.A., Al-Selwey, W.A., Abdela, K.A., Alghamdi, S.S., Mohammed, N.A., Refay, Y.A., Migdadi, H.M., Al-Somain, B.H., Muharram, A.A., Al-Selwey, W.A., Abdela, K.A., Alghamdi, S.S., Farooq, M., 2019. *Agro-morphological characterization of lentil genotypes in dry environments.* Int. J. Agric. Biol. Farooq, 22: 1320-1330. https://doi.org/10.17957/IJAB/15.1204

Piepho, H.P., Möhring, J., 2007. *Computing heritability and selection response from unbalanced plant breeding trials.* Genetics, 177: 1881-1888. https://doi.org/10.1534/genetics.107.074229

Pouresmael, M., Zahravi, M., Ghanbari, A.A., 2018. *Evaluation and characterization of ICARDA elite germplasm of lentil.* Iran J. Genet. Plant Breed, 7: 9-23. https://doi.org/10.30479/ijgpb.2019.9000.1199

Pushpavalli, R., Zaman-Allah, M., Turner, N.C., Baddam, R., Rao, M.V., Vadez, V., 2015. *Higher flower and seed number leads to higher yield under water stress conditions imposed during reproduction in chickpea.* Funct. Plant Biol., 42: 162-174. https://doi.org/10.1071/FP14135

Rehman, A.U., Amjad Ali, M., Atta, B.M., Saleem, M., Abbas, A., Mallahi, A.R., 2009. *Genetic studies of yield related traits in mungbean (Vigna radiata L. Wilczek).* Aust. J. Crop Sci., 3: 352-360.

Russu, F., Porumb, I., Mureşanu, F., Tritean, N., 2019. *Estimation of the variability and inheritance of*

some quantitative traits in two rows spring barley in the conditions of ARDS Turda. Rom. Agri. Res., 36: 67-77.

Schmidt, P., Hartung, J., Rath, J., Piepho, H., 2019. *Estimating broad-sense heritability with unbalanced data from agricultural cultivar trials*. Crop Sci., 59(2): 525-536.
https://doi.org/10.2135/cropsci2018.06.0376

Sharma, V., Kumar Paswan, S., Kumar Singh, V., Khandagale, S., 2014. *Correlation and path coefficient analysis of economically important traits in lentil (Lens culinaris Medik.) germplasm*. The Bioscan, 9: 819-822.

Shrestha, R., Turner, N.C., Siddique, K.H.M., Turner, D.W., 2006. *Physiological and seed yield responses to water deficits among lentil genotypes from diverse origins*. Aust. J. Agric. Res., 57: 903-915.
https://doi.org/10.1071/AR05204

Singh, M., Kumar, S., Basandrai, A.K., Basandrai, D., Malhotra, N., Saxena, D.R., Gupta, D., Sarker, A., Singh, K., 2020. *Evaluation and identification of wild lentil accessions for enhancing genetic gains of cultivated varieties*. PLoS One, 15: 1-14.
https://doi.org/10.1371/journal.pone.0229554

Singh, S.S., Kumar, A., Kumar, S., 2018. *Heritability of yield and its attributing traits in lentil (Lens culinaris Medic)*. Int. J. Chem. Stud., 4: 61-64.

Sonnante, G., Hammer, K., Pignone, D., 2009. *From the cradle of agriculture a handful of lentils: History of domestication*. Rend. Lincei, 20: 21-37.

Vanda, M., Khodambashi, M., Houshmand, S., Shiran, B., Amiri-Fahlian, R., 2013. *Determination of gene action and heritability for some biometrical traits in lentil (Lens culinaris Medik.) Using F 2:3 families*. Int. J. Agric. Crop Sci., 5: 1427-1431.

Whitehead, S.J., Summerfield, R.J., Muehlbauer, F.J., Coyne, C.J., Ellis, R.H., Wheeler, T.R., 1980. *Crop improvement and the accumulation and partitioning of biomass and nitrogen in lentil*. Crop Sci., 40: 110-120.

Yuan, H.Y., Saha, S., Vandenberg, A., Bett, K.E., 2017. *Flowering and growth responses of cultivated lentil and wild Lens germplasm toward the differences in red to far-red ratio and photosynthetically active radiation*. Front Plant Sci., 8: 1-10.
https://doi.org/10.3389/fpls.2017.00386

Zhang, H., Pala, M., Oweis, T., Harris, H., 2000. *Water use and water-use efficiency of chickpea and lentil in a Mediterranean environment*. Aust. J. Agric. Res., 51: 295-304.
https://doi.org/10.1071/AR99059