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GENETIC VARIATION IN LENTIL GENOTYPES BY MORPHO-AGRONOMIC TRAITS AND RAPD-PCR

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ABSTRACT

In Iraq, one of the most common problems in lentil crop is the narrow genetic background, which must be explored to increase the production of lentil. Agro-morphological and molecular characterization were performed to evaluate the genetic diversity among nine lentil genotypes. Lentil genotypes were planted during crop season 2014/2015.According to statistical analysis, the lentil genotypes significantly differed in all studied traits. Based on phenological performance, genotypes Sulaimani-1, Sulaimani-2, and Sulaimani-3 were the genotypes that need the maximum number of days for 50% flowering and physiological maturity. Genotype FLIP 2007-69L excelled other genotypes for pod weight and seed yield. Based on the scatter plot of all agro-morphological data, PC1 (F1) and PC2 (F2) scores computed for 44.338 and 22.200% of the total variance, respectively. According to ascending hierarchical clustering, the analysis revealed three major clusters. Eighteen RAPD primers amplified 92 polymorphic bands. Primer OPA10showed the highest number of polymorphic fragments (8), whileOPA09 produced the lowest (1).Maximum gene diversity (0.840) and PIC (0.819) were found at the loci NOCOD04, OPA10, and OPN07 while the minimum gene diversity (0.444) and PIC (0.346) observed at the locus OPA09.According to dendrogram and PCA, nine genotypes were discriminated into three main clusters. The lowest dissimilarity value was 0.313 (between FLIP 2006-20L and FLIP 2007-69L), whereas the highest distance (0.803) was between FLIP 2007-53L and Sulaimani-2. AMOVA indicated the high variation (60%) within lentil genotypes; however, considerable variation (40%)was recorded between Local and ICARDA populations.

Keywords: Genetic diversity, Clustering, Agro-morphological traits, RAPD marker.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is designed as one of the oldest legumes. It is a self-pollinated annual plant and belonged to Fabaceae family. Lentil contains 14 chromosomes and has a huge genome size (4063 Mbp). Lentil is largely cultivated in Middle East, North Africa, North America and Australia (Arumuganathan and Earle, 1991). FAO-STAT demonstrated that the world lentil production for the year 2013 was 4.9 million tons primarily producing in Canada, India, and Turkey(FAOSTAT, 2015). Lentil was cultivated as human food and feed. It is rich in protein (22-35%), carbohydrates (55-60%), energy, fiber, vitamin A, vitamin B, potassium, iron, and zinc (Tickoo *et al*., 2005). Evaluation of genetic variability among genotypes presenting in Iraq is to the discernment of the available genetic variation and potential use for breeding program. Previously, the researchers were relied on morphological data for clustering the set of lentil genotypes. The knowledge of genetic distance among genotypes and association of some traits with yield are an important point for a breeder for improving quantitative characters (Erskine and Witcombe, 1984). In spite of morphological markers which has an act in the selection of favorable gene(s) in plant breeding programs, further validation at the level of molecular markers is necessary.

In view of this point, it was hypothesized that molecular biology tools could be helpful to the development of DNA markers that can be used for genetic mapping and diversity estimation (Soller and Beckmann, 1983; Kumar *et al*., 2011). Several methods of DNA-based markers have been used in genotyping of lentil like inter-simple sequence repeat (ISSR) (Zaccardelli *et al*., 2012; Joshi *et al*., 2013), simple sequence repeat (SSR) markers (Liu *et al*., 2008; Kaur *et al*., 2011), amplified fragment length polymorphism (AFLP) analysis (Toklu *et al*., 2009), restriction fragment length polymorphism (RFLP) (Havey *et al*., 1989), Sequence-related amplified polymorphism (SRAP) (Bermejo *et al*., 2014) and random amplified polymorphic DNA (RAPD) (Afzal *et al*., 2004).

RAPD is a simple, easy, friendly and faster molecular marker technique. RAPD is a dominant marker and considered as a great potential fingerprinting method and gave a high level of polymorphisms. RAPD technique is not in need of the knowledge of gene sequence and DNA probes (Tosti and Negri, 2002; Afzal *et al*., 2004). This method has been widely used for determination of relationship among and between genotypes (Xu *et al.*, 2000; Ba *et al*., 2004). Therefore,

the present research was planned with the objectives to determine the:

a. genetic variation among the lentil genotypes based on morpho-agronomic traits, and
b. genetic dissimilarity between lentil groups (ICARDA and Local groups) and within groups based on clustering and RAPD data.

MATERIALS AND METHODS

Plant materials: Lentil (*Lens culinaris* M.) genotypes employed in this study obtained from two origins. FLIP 2006-20L, FLIP 2007-53L, FLIP 2007-69L, FLIP 04-63L, FLIP 05-14L and FLIP 04-50Lwere taken from International Center forAgricultural Research in Dry Areas (ICARDA),Syria and three local genotypes (Sulaimani-1, Sulaimani-2, and Sulaimani-3) were obtained from Agricultural Research Station, Bakrajow (ARSB), Iraq (Table 1).

Field experiment: Seeds of different genotypes were planted and grown under rainfed conditions at the farm of Bakrajow Experimental Research Station, Sulaimani, Iraq with latitude 35°32'30"N, longitude 45°21'00"E and 738 masl (Metre above sea level). Randomized complete blockdesign (RCBD) layout with three replicationswas used. Regarding of land and weed management, the soil was plowed and harrowed once before making the plots. Seeds (48 kg/ha and 28.8 g/plot) were planted on November 14, 2014, by hand. The area of the experimental plot was 3 x 2 m having four rows. The distance between each two rows, two plants, and two plots were 40, 8 and 50 cm, respectively. Seven kilogram fertilizer (8-15-15) (Nitrogen-Phosphate-Potassium) was applied during sowing. Control of weeds was carried out manually on all the plots at 5 and 10 weeks after sowing.

Collection of data: Data of agro-morphological traits were collected following Diversity International Guidelines (Cristobal *et al.,* 2014). Ten randomly plants per replication were taken for measuring of traits except biological yield, harvest index and seeds yield which were measured by whole plot. The measured traits are: plant height (PH), number of days to 50% flowering (NDF), number of days to physiological maturity (NDPM), grain filling period (GFP), number of pods per plant (NPO), number of seeds per pod (NSPO), pods weight per plant (PW), number of seeds per plant (NSP), hundred seeds weight (HSW), biomass yield per plot (BY), seeds yield per plot (SY) and harvest index (HI%).

Quantification of pigment: The leaf pigment (chlorophyll *a, b* and carotenoid) was extracted and calculated according to the protocol of Lichtenthaler *et al.* (2001). Chlorophyll *a, b* and carotenoid contents in extracts were measured by a spectrophotometer using absorbance 663, 646 and 470 nm.

Seed oil content (%): Dry seeds were grained using a pestle and mortar. Three gram of each grained sample was placed into three tubes. For oil extracting, two hundred mL of hexane was added to the tubes and placed in the Soxhlet apparatus for 2 hours. The temperature wasattended to 69ºC. The samples were dried at ambient air temperature for four days. The oil content was calculated from the weight of oil and the mass of seeds:

Seed ash content (%): To determine the total ash, 5 gram of ground samples of different lentil genotypes were put into a porcelain crucible and placed in a muffle furnace at 525ºC for three days (AOAC, 1990). After three days, the porcelain crucible was cooled and weighed.

Genomic DNA extraction: DNA was isolated from 20 day-old plants. The collected leaves were frozen and ground in liquid nitrogen. DNA was extracted using CTAB method (Doyle and Dickson, 1987).

Polymerase chain reaction (PCR) and gel electrophoresis: The PCR reaction (20 µL) contained 4 µL template DNA, 3 µL of each primer (20 µM) and 10 µL master mix (1× PCR mix, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1 U Taq polymerase) (Sinaclon). The twenty-seven RAPD primers (Sinaclon) tested are presented in Table 2. Amplifications were carried out in a Biometra gradient thermocycler with this platform: an initial denaturation at 95°C for 5 min, followed by 40 cycles of 1 min at 94°C, 1 min at 35, 36, 37, 40 and 42°C (depending on the RAPD markers as shown in Table 2) and 2 min at 72°C, with a final extension for 7 min at 72°C.PCR products were mixed with loading buffer and separated on a 1.6% (w/v) agarose gel (containing a 0.4 µg/mL ethidiumbromide) in 1X TBE (Sinaclon) at 80 V for 1hr and 40 min. DNA Bands were visualized on UV-transil luminator and photographed. The sizes of the DNA bands were calculated depending on DNA ladder of a 1kb (Sinaclon).

Statistical analysis: For agro-morphological data, the software XLSTAT version 11 was used for statistical analysis like one-way analysis of variance (ANOVA-RCBD) pair-wise comparison (LSD), principal component analysis (PCA) and hierarchical cluster analysis by using the Euclidean distance and Ward's clustering method (Eriksson *et al.*, 1999). For molecular analysis, Jaccard coefficient (Jaccard, 1908), dissimilarity matrix, principal component analysis (PCA) and hierarchical cluster analysis was measured based on binary data (0 and 1) by using XLSTAT version 11. Polymorphism information content (PIC), and gene diversity were computed by using Power marker software, version 3.25. Mantel test (Mantel, 1967) and molecular variance among and within populations (Peakall *et al.,* 2012) were performed by GenAlEx software.

RESULTS AND DISCUSSION

Agro-morphological performance: Providing the necessary information for the management of population in the genebank and estimation of the degree of diversity of samples was the objective of this characterization. Sulaimani-3 was being the tallest (48.763 cm) followed by FLIP 05-14L (45.623 cm) while the lowest plant stature value was recorded in FLIP 2007-53L (41.913 cm) and Sulaimani-2 (41.913 cm) (Table 3).Mondal *et al.* (2013) revealed a range of plant height from 37.1 to 50.2 cm among six lentil genotypes. Our result of plant height was greater that this detected by Mekonnen *et al.* (2014) who stated a range of plant height from 32.09 to 35.37 cm among three populations of lentil. These differences may be due to their variations in genetic makeup and climatic factors.

Analysis of pairwise comparison showed that the number of days required to 50% flowering, physiological maturity and grain filling period varied significantly among lentil genotypes (Table 3). Among the studied genotypes, the minimum days to 50% flowering (136.118 days) were observed in FLIP 05-14L which had not a significant difference with FLIP 2007-53L and FLIP 04-63L. In contrast, Sulaimani-2 took maximum days to flowering (158.135 days), which differed significantly from Sulaimani-1 (155.125 days). This difference in the results of phenological traits may be explained by the variation between environmental factors and genotypes. Lázaro *et al.* (2001) reported a low number of days to 50% flowering with 117.8 days in lentil in Spain. Piergiovanni (2000) reported this period ranging from 104 to 106 days in the Italians lentil populations. Sulaimani-3 took a maximum number of days to physiological maturity (200.166 days). In contrast, FLIP 2007-69L took the lowest number of days to maturity (187.166 days) followed by FLIP 04-63L. These results indicated that the local genotypes need more days to flowering and maturity compared with the ICARDA genotypes. These differences may be due to their variations in genetic makeup and climatic factors. This part of results is in agreement with the results of Bicer and Şakar (2007) that showed ICARDA genotypes need less number of days to flowering and maturity than local genotypes. Concerning the grain filling period, FLIP 05-14L (55.381 days)was lasted among the other genotypes whereas Sulaimani-2 (42.031 days) was the earliest. Similar results of the variability among lentil genotypes for days to flowering and physiological maturity were demonstrated by Chakraborty and Haque (2000) and Mondal *et al.* (2007, 2013).

To summarize the data of yield components, ANOVA and pairwise comparison (LSD) were carried out. The results of yield components traits displayed high significant variations for all traits of yield component except the weight of pods and number of seeds per plant which showed significant differences among genotypes (Table 3). The presence of such variation performs selection to be used as parents in programs of breeding. According to results of the pair-wise comparison (Table 3), there were significant differences among 9 lentil genotypes for all traits of yield components. The most important attribute, the number of pods per plant had shown a significant difference and a wide range of variability among genotypes. FLIP 04-63L produced the minimum number of pods per plant (64.333). WhilstFLIP 05-14L produced a maximum number of pods (91.433) followed by Sulaimani-3 (89.600). Relating the number of seeds per pod, Sulaimani-1 and Sulaimani-2 noticed the highest numbers of seeds per pod (1.345 and 1.231, respectively). FLIP 05-14L revealed lower seed number per pod (0.910). These results were consistent with the results reported by Mekonnen *et al.* (2014) who found a range of number of seeds per pod from 1.169 to 1.355 among lentil populations.

Maximum pods weight per plant (4.712 g) was observed in FLIP2007-69L, which was statistically similar to FLIP 2006-20L (4.127 g). The genotypes Sulaimani-3 and Sulaimani-1recorded the highest number of seeds per plant (105.567 and 103.533, respectively), while FLIP 04-50Lobtained the highest 100 seeds weight (4.357 g) pursued by FLIP 05-14L (4.007 g). FLIP 2006-20L demonstrated minimum number of seeds per plant (73.967). The lowest 100 seeds weight was shown by Sulaimani-3 (3.107 g). As shown in Table 3, Sulaimani-1 and FLIP2007-69L gave high values of biomass yield (3927.113g) and seeds yield (1112.55 g) per plot, respectively, while the lowest values of biomass yield and seeds yield was displayed by FLIP 2007-53L (2266.590 g) and FLIP 04-50L (644.007 g), respectively. The highest seeds yield in FLIP 2007-69L is due to high translocation of dry matter partitioning to reproductive organs. The lowest seeds yield in FLIP 04-50L (644.007 g) may be due to less number of filled pods and number of false pods per plant.The result also exhibited that FLIP 05-14L had a large number of pods per plant, but the low quantity of seeds yield (762.610 g). This result might be due to number of false pods per plant. As noticed in Table 3, the local genotypes have large quantities of biomass yield compared to ICARDA genotypes. The reason for this result is that the local genotypes have along vegetative period and a large amount of primary branches, secondary branches, leaves, and leaflets compared to ICARDA genotypes. As indicated in Table 3, harvest index demonstrated a wide range of variability. FLIP 2007-69L recorded the highest harvest index (34.205%). On the other hand, Sulaimani-2 took the lowest quantity of harvest index (19.807%). In conclusion, the results demonstrated that the mean of seeds yield of FLIP 2007-69L was overcoming the mean of local lentil yield. Our results of biological and seed yields were higher than those obtained by Cristobal *et*

al.(2014) who observed a significant difference among lentil genotypes in term of biological yield (160.00 – 1120.00 g) and seeds yield (40.24 – 476.20g). This difference between two results may be due to use of different genotypes.

Leaf pigment like chlorophyll *a*, *b* and carotenoid was estimated in nine genotypes. The content of chlorophyll *a, b* and carotenoid ranged from 7.578 to 13.410, 2.804 to 4.417 and 740.004 to 1317.697 µg/mL, respectively (Table 3). The minimum quantity of chlorophyll *a* (7.578 µg/mL), *b* (2.804 µg/mL) and carotenoid (740.004 µg/mL) was exhibited by FLIP 2007-69L, while Sulaimani-2 gave the maximum amount of chlorophyll *a* (13.41 µg/mL), b (4.417 µg/mL) and carotenoid (1317.697 µg/mL).The oil and ash contents were varied from 3.548 to 6.341% and 1.875 to 2.423%, respectively (Table 3). The high quantity of seed oil (6.341%) and seed ash (2.423%) content was reported by Sulaimani-2 while the lowest values of seed oil (3.548%) and seed ash (1.875%) contents inscribed by Sulaimani-1 and FLIP 2007-53L. Wang and Daun (2004) reported 0.243 and 0.302 mg/mL of seed protein content in lentil. Ash content was shown to be in the range of 2.39-2.89% by Wang *et al.* (2009). Oil content in our study was higher than those described by Alghamdi *et al.* (2014) which ranged from 0.79 to 1.19%.

The two principal components PC1 and PC2 revealed 66.538% of the original phenotypic variation. The value of the contribution of first and second components was: 44.338and 22.200%, respectively (Figure 1).The most important component, PC1, was positively and negatively influenced by characters: number of days to 50% flowering (0.350), number of days to physical maturity (0.344), grain filling period (-0.305), hundred seeds weight (-0.251), biomass yield per plot (0.280) and seed ash content (0.234). The main characteristics of component 2 (PC2) contributing to the total variation were pods weight (0.280), a number of seeds per plant (0.310), seeds yield per plot (0.378), leaf chlorophyll *a* and *b* contents (-0.334 and -0.291) and leaf carotenoid content (-0.328). The third component (PC3) was influenced by plant height (0.392), the number of pods per plant (0.476), the number of seeds per pod (-0.387), harvest index (-0.316) and seed oil content (-0.387) (Table 4).

As indicated in Figure 1, genotype FLIP 2007-69L and Sulaimani-2 were the most distant genotypes from the rest of genotypes. The two local genotypes Sulaimani-1 and Sulaimani-3 were more similar. Concerning the genotypes of ICARDA, we observed that the most of the genotypes were dispersed on the plot. This result indicates that there is a large variation among ICARDA genotypes. Bicer and Sakar (2007) have found a significant variation in the agronomic characteristics between some of the lentil landraces in Turkey. Bacchi *et al.* (2010) used principal component analysis for morphological and agronomic data to allow the discrimination of lentil genotypes and they found that Laird variety was far from all the other genotypes. In conclusion, according to of scatter plot, there was a high level of phenotypic variation. The genotypes (FLIP 2007-69L and Sulaimani-1) have superior for a trait like seed yield which could be utilized in the breeding program and used as a parent in some crosses.

A dendrogram constructed to estimate genetic relatedness based on agro-morphological data by using Euclidean distance (Table 5 and Figure 2). The dissimilarity value was varied from 148.988 (between FLIP 05-14L and FLIP 2006-20L) to 1685.211 (between FLIP 2007-53L and Sulaimani-1). Clustering analysis revealed three major groups (Figure 2). Cluster or group I comprised the local genotypes: Sulaimani-1, Sulaimani-2, and Sulaimani-3. FLIP 2007-53L and FLIP 04-63L belonged to cluster II. FLIP 2007-69L, FLIP 2006-20L, FLIP 05-14L and FLIP 04-50L formed the last cluster. As presented in Figure 2, the result marked that there is high variability among ICARDA genotypes compared to local genotypes. Mekonnen *et al.* (2014) clustered 228 lentil genotypes into six major classes. Previous literature reported two cluster analyzes among 27 lentil landraces using the Ascending hierarchical clustering analysis (Cristobal *et al*., 2014).

Molecular analysis of RAPD

Alleles variation: Out of 27 primers, 18 primers were considered informative for the purpose of resolving genetic marker differences among and within lentil populations (Table 6). Eighteen RAPD primers produced 133 amplified products with the average of 7.389 bands per primer across all genotypes. The size of bands ranged from 250 to 2500 bp. The most informative primer was NOCOD04, which revealed the highest amplified bands (12 bands), whereas OPA09 produced the lowest number (2 bands). This difference may be due to the diversity of genotypes and the selection of RAPD primers with measurable bands. The eighteen selected primers gave the high-intensity bands with minimal smearing and sufficient variation among different genotypes. Only 2 primers (OPJ12 and OPM06) were didn't scored polymorphic bands among lentil genotypes. The eighteen primers are amplified 92 polymorphic (69.173%) with an average of 5.111 bands per primer. The number of polymorphic bands varied from 1 to 8. RAPD primer OPA10 gave the highest number of polymorphic fragments (8 fragments), and primer OPA09 produced the lowest number of polymorphic bands (1 band). The primer has a high ability of recognition, that is; the primer has the ability to give the highest number of polymorphic bands according to the total number of variation. The polymorphisms were accounted according to the presence and absence of fragments. The absence of bands may be due to the failure of primers to anneal at a

site in some individuals due to nucleotide sequence differences or by insertions or omission between primer locations (Clark and Lanigan, 1993). The difference in a number of polymorphic bands might be due to the amount of GC content of the primers used in this study.

The variation in the number of bands amplified bydifferent primers also is influenced by variable factors like primer sequence and a low number of annealing loci in the genome. The polymorphic ratio indicates the presence of different alleles in lentil genotypes, suggesting the existence of reasonable variation between these genotypes. Rana *et al*. (2007) studied 29 lentil cultivars and landraces; they obtained 42.3% polymorphic bands using 72 RAPD and 25 STMS. Tayyeba (2003) found 50% polymorphic bands among lentil varieties. They revealed that the differences could be caused by the sequence of primers used and the genotypes evaluated. Alabboud *et al*. (2009) used 4decamer primers that produced 43 RAPD markers, 27 of them were polymorphic (62.7%) among six lentil varieties. Ganjali *et al.* (2012) used random primers (RAPD) in lentil to produce a total of 187 bands, 133 bands of them (61.4%) were polymorphic fragments. They characterized amplified fragments ranged from 200-5000 bp.The researchers also found that the number of amplified bands varied from 6 (OPN06) to 36 (OPN14), with an average of 20.77 bands per primer.

Estimation of PIC, gene diversity, and unique bands: As shown in Table 7, maximum gene diversity (0.840) and PIC (0.819) were at the loci NOCOD04, OPA10, and OPN07. Among the 18 primers, the lowest gene diversity (0.444) and PIC (0.346) revealed at the locus OPA09. Regarding all markers, the mean of gene diversity and PIC were 0.741 and 0.704, respectively. The high estimation of gene diversity and PIC due to the fact that most of the alleles were present at high frequencies. The high PIC values reflected the power of RAPD primers to assess genetic diversity among lentil genotypes. The high level of gene diversity suggests that gene pool of lentil has a high level of allelic polymorphism that could be exploited for a breeding program of lentil. The values of gene diversity and PIC in this study were higher than those obtained by Ganjali *et al*. (2012). The researchers found the mean of gene diversity with 0.198 and PIC with 0.269 in lentil genotypes.

Unique bands are referred as markers that identify genotypes from the others by their presence or absence. The bands that are present in one genotype but not found in the others are defined as unique positive markers, opposite to the unique negative markers. Lentil genotypes were characterized by 8 positive and 6 negative unique bands as shown in Table 7. The primer OPN06 had the highest number (3) of unique RAPD markers (1 positive and 2negatives). The existence of unique band in some genotypes might be due to thedifference in origin before domestication. These results indicated that these loci can be used to analyze the genetic diversity and to the identification of genotypes. El-Nahas *et al*. (2011) found 13 positive and negative unique bands among six lentil genotypes by using six ISSR primers. They showed that primer HB11 had the highest number of unique bands (5).

Principle component analysis and genotypes clustering: The variability among lentil genotypes was also estimated by principal component analysis. The analysis basing on RAPD data produced Eigenvalues of each principal component axis. The first two principal axes displayed 50.54% of total variation describing 9 lentil genotypes. The high value of components indicates favorable sampling of genotypes grouping and primers from the genome regions. The value of eigenvectors calculated for the nine lentil genotypes produced similar results to that obtained by dendrogram based on Jaccard's coefficient. According to PCA plot, nine genotypes were discriminated into three main clusters (Figure 3). The first main cluster included all genotypes except local genotypes (Sulaimani-1, Sulaimani-2, and Sulaimani-3) which grouped into two different clusters. The results obtained from PCA indicated a high variation among lentil genotypes. By using PCA method, Ganjali *et al*. (2012) found 26.35% of the total variations were explained by three components using the RAPD markers.

Genetic relationship through UPGMA: The genetic distance was assessed through 92 alleles from 18 RAPD scored markers (Table 8). The lowest dissimilarity value was 0.313 (between FLIP 2006-20L and FLIP 2007-69L) which means the presence of high similarity between these two genotypes. These two genotypes might be introduced and constructed from the same region and parents. The highest distance was 0.803 which was between FLIP 2007-53L and Sulaimani-2. This result means that the similarity between them is very low, and these genotypes might be constructed and collected from different parents and origins. The difference between the highest and lowest dissimilarity values indicate the high variation among the 9 genotypes. The data of 92 bands are converted to dissimilarity matrix. A dendrogram was created based on the Jaccard's coefficient to evaluate the level of the relationship between genotypes (Figure 4). According to the dendrogram, nine genotypes were discriminated into three main clusters. The first main cluster included ICARDA genotypes. This cluster was contained four sub-clusters. The first sub-cluster comprised FLIP 05-14L and FLIP 04-50L while the second sub-cluster included FLIP 2006-20L and FLIP 2007-69L. FLIP 2007-53L and FLIP 04-63L were formed the third and fourth sub-cluster, respectively. Sulaimani-2 and Sulaimani-3 were grouped in the second cluster while the last group of clustering included genotype Sulaimani-1. Sulaimani-1 was more distant from other

genotypes.The results of dendrogram showed that different levels of genetic dissimilarity presented between genotypes that coming from same or different origins. By using a phylogenetic tree, Hoque *et al.* (2012) clustered six genotypes of lentil into two main groups by using RAPD data. In their study, they found a range of genetic distance from 0.069 to 0.500 among six lentil genotypes. Alabboud *et al*. (2009) grouped six lentil cultivars into two main clusters by using RAPD method.

Analysis of molecular variance (AMOVA): The data of table 9 showing the analysis of molecular variance among populations (Local and ICARDA) and within apopulation by using RAPD data. AMOVA exposed significant genetic distance between the two populations of lentil (Local and ICARDA) and within a population (P-value = 0.02). The results exhibited that all examined genotypes were different RAPD phenotype. The estimated variances between lentil populations were 9.952 and within the population were 14.857 out of 24.810. The variation among the lentil populations (40%) was lower than within populations (60%). This result of AMOVA indicated that high variation within a population (among genotypes of the population) was observed in lentil populations, but considerable variation between populations still exists. This result suggests that gene flow between lentil genotypes from different locations in Kurdistan is rather limited. Tewari *et al*. (2015) employed RAPD markers to calculate AMOVA among lentil genotypes. They found a variation of 36.8% between lentil populations and 63.2% of the population.

Correlation and comparison between molecular and morphological data: The relation between the genetic distance matrices of RAPD markers (Table 8) and Euclidean distance (Table 5) depending on agro-morphological traits was calculated by Mantel test. The Mantel test revealed a significant correlation (r=0.472*, P≤0.019) between two matrices. The significant correlation indicates that these independent data reflect the same pattern of genetic diversity and validate the use of these data to estimate the disparate dissimilarity for morphological traits in the lentil genotypes. Therefore, clustering of lentil genotypes derived with RAPD data was consistent with that obtained with agro-morphological data. Based on the Mantel test, it is known that the genes controlling morphological characters are illustrated by RAPD analysis. The dendrogram developed from agro-morphological data (Figure 2) demonstrated that the lowest dissimilarity was between Sulaimani-2 and Sulaimani-3. Similar results were observed from the dendrogram of molecular data (Figure 4). The dendrogram of morphological and molecular data (Figures 2 and 4) showed the highest similarity between FLIP 05-14L and FLIP 04-50L. The two dendrograms grouped FLIP 2007-53L and FLIP 04-63L in the same cluster. These results indicated that the results of two dendrograms are very similar.

Table 1. Name, pedigree and origin of nine lentil genotypes used in this study.

Genotype name	Pedigree	Origin
FLIP 2006-20L	ILL 5883 X ILL 590	ICARDA
FLIP 2007-53L	ILL 590 X ILL 7723	ICARDA
FLIP 2007-69L	ILL 590 X ILL 5769	ICARDA
FLIP 04-63L	ILL 358 X BL X 84135	ICARDA
FLIP 05-14L	ILL 5883 X ILL 6475	ICARDA
FLIP 04-50L	ILL 7005 X ILL 1939	ICARDA
Local: Sulaimani-1	-	ARSB
Local:Sulaimani-2	-	ARSB
Local:Sulaimani-3	-	ARSB

Table 2. Name, sequence, and an annealing temperature of RAPD primers.

No.	Name of primer	Sequence from 5 to 3	Annealing temperature (°C)
1	NOCOD04	GACCGACCCA	36
2	OPA02	GTGAGGCGTC	35
3	OPA04	AATCGGGCTG	35
4	OPA05	AGGGGTCTTG	35
5	OPA07	GAAACGGGTG	35
6	OPA09	GGGTAACGCC	35
7	OPA10	GTGATCGCAG	35
8	OPA13	CAGCACCCAC	35
9	OPC05	GATGACCGCC	36
10	OPC11	AAAGCTGCGG	36
11	OPF03	CCTGATCACC	36
12	OPG09	TGAGCCTCAC	36
13	OPJ01	CCCGGCATAA	35
14	OPJ06	TCGTTCCGCA	35
15	OPJ12	GTCCCGTGGT	36
16	OPJ19	GGACACCACT	36
17	OPM06	CTGGGCAACT	36
18	OPN06	GAGACGCACA	36
19	OPN07	CAGCCCAGAG	35
20	OPN16	AAGCGACCTG	36
21	OPN20	GGTGCTCCGT	35
22	OPP19	GGGAAGGACA	35
23	OPR11	GTAGCCGTCT	42
24	OPR12	ACAGGTGCGT	37
25	OPr10	AGGCGGGTAC	40
26	OPV03	CTCCCTGCAA	37
27	OPW18	TTCAGGGCAC	36

Table 3. Mean and pair-wise comparison (LSD) of agro-morphological characters in nine lentil genotypes.

Traits	G1	G2	G3	G4	G5	G6	G7	G8	G9	$LSD_{(0.05)}$
PH	42.757^{b}	41.913^{b}	44.607^{b}	41.957^{b}	44.800^{b}	41.953^{b}	45.623^{ab}	48.763^{a}	45.543^{ab}	3.743
NDF	140.125^{c}	136.125^{d}	140.125^{c}	136.135^{d}	155.125^{b}	158.135^{a}	136.118^{d}	155.125^{b}	140.142^{c}	0.025
NDPM	190.833^{c}	189.833^{c}	187.166^{d}	187.499^{d}	197.833^{b}	200.166^{a}	191.499^{c}	200.166^{a}	190.499^{c}	1.999
GFP	50.708^{b}	53.708^{a}	47.041^{c}	51.364^{b}	42.708^{d}	42.031^{d}	55.381^{a}	45.041^{c}	50.358^{b}	2.003
NPO	70.600^{de}	76.933^{bcde}	80.933^{abcd}	64.333^{e}	79.467^{abcd}	73.200^{cde}	91.433^{a}	89.600^{ab}	84.800^{abc}	13.082
NSPO	1.050^{cd}	1.100^{bcd}	1.186^{bc}	1.163^{bc}	1.345^{a}	1.231^{ab}	0.910^{e}	1.176^{bc}	0.992^{de}	0.137
PW	4.127^{ab}	3.604^{bc}	4.712^{a}	3.432^{bc}	3.255^{bc}	2.894^{c}	3.019^{c}	3.624^{bc}	3.694^{bc}	0.966
NSP	73.967^{c}	85.067^{bc}	92.200^{abc}	74.367^{c}	103.533^{ab}	92.300^{abc}	77.167^{c}	105.567^{a}	86.533^{bc}	18.795
HSW	3.897^{b}	3.340^{c}	3.890^{b}	3.750^{b}	3.113^{c}	3.137^{c}	4.007^{b}	3.107^{c}	4.357^{a}	0.315
BY	2999.593^{bcd}	2266.590^{d}	3251.027^{abc}	2700.017^{cd}	3927.113^{a}	3522.380^{ab}	3078.763^{bc}	3589.610^{ab}	3121.140^{bc}	734.323
SY	826.737^{b}	772.483^{bc}	1112.550^{a}	877.143^{b}	1027.733^{a}	696.567^{cd}	762.610^{bcd}	764.030^{bc}	644.007^{d}	119.809
HI	27.966^{bcd}	33.949^{ab}	34.205^{a}	32.317^{abc}	26.338^{cde}	19.807^{f}	25.991^{def}	21.543^{ef}	20.753^{ef}	6.215
CHA	10.631^{e}	10.480^{e}	7.578^{g}	11.481^{c}	11.826^{b}	13.410^{a}	10.221^{f}	11.196^{d}	11.977^{b}	0.171
CHB	3.865^{b}	3.596^{c}	2.804^{e}	3.681^{c}	4.005^{b}	4.417^{a}	3.419^{d}	3.915^{b}	3.881^{b}	0.146
CA	1052.659^{e}	1024.048^{f}	740.004^{h}	1072.857^{d}	1153.091^{b}	1317.697^{a}	946.262^{g}	1051.609^{e}	1135.202^{c}	13.464
SOC	4.523^{bcd}	4.601^{bc}	4.882^{b}	6.123^{a}	3.548^{d}	6.341^{a}	3.708^{cd}	4.574^{bcd}	3.812^{cd}	1.051
SAC	2.107^{bc}	1.875^{c}	2.319^{ab}	2.333^{ab}	2.384^{ab}	2.423^{a}	2.217^{ab}	2.363^{ab}	2.219^{ab}	0.313

G1: FLIP 2006-20L, G2: FLIP 2007-53L, G3: FLIP 2007-69L, G4: FLIP 04-63L, G5: Sulaimani-1, G6: Sulaimani-2, G7: FLIP 05-14L, G8: Sulaimani-3 and G9: FLIP 04-50L. PH: Plant height (cm), NDF: Number of days to 50% flowering, NDPM: Number of days to physiological maturity, GFP: Grain filling period (days), NPO: Number of pods per plant, NSPO: Number of seeds per pod, PW: Pods weight per plant (g), NSP: Number of seeds per plant, HSW: 100 seeds weight (g), BY: Biomass yield (g) per plot, SY: Seeds yield (g) per plot, HI: Harvest index (%) per plot, CHA: Chlorophyll *a* (µg/mL), CHB: Chlorophyll *b* (µg/mL), CA: Carotenoids (µg/mL), SOC: Seed oil content (%), SAC: Seed ash content (%).Means connected with the different letters signify a significant difference between means at P≤0.05 according to Fisher's LSD.

Table 4. Eigenvector of different agro-morphological traits in nine lentil genotypes.

Traits	F1	F2	F3
PH	0.089	0.308	0.392
NDF	0.350	0.107	-0.046
NDPM	0.344	0.023	0.091
GFP	-0.305	-0.183	0.188
NPO	0.030	0.242	0.476
NSPO	0.220	0.181	-0.387
PW	-0.200	0.280	-0.120
NSP	0.255	0.310	0.025
HSW	-0.251	-0.056	0.214
BY	0.280	0.260	0.065
SY	-0.071	0.378	-0.319
HI	-0.271	0.103	-0.316
CHA	0.265	-0.334	0.037
CHB	0.287	-0.291	0.024
CA	0.271	-0.328	-0.013
SOC	0.053	-0.191	-0.387
SAC	0.234	0.171	-0.076

Table 5. Dissimilarity matrix (Euclidean distance) among 9 lentil genotypes based on agro-morphological data.

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9
G1	0.000								
G2	735.719	0.000							
G3	493.134	1079.627	0.000						
G4	304.606	448.871	685.914	0.000					
G5	955.029	1685.261	797.183	1239.578	0.000				
G6	601.171	1292.288	762.390	877.570	548.468	0.000			
G7	148.988	816.168	441.810	416.447	913.330	583.517	0.000		
G8	594.841	1323.835	577.969	898.310	440.346	283.541	522.925	0.000	
G9	235.371	871.436	626.784	486.137	893.222	444.679	227.522	491.531	0.000

G1: FLIP 2006-20L, G2: FLIP 2007-53L, G3: FLIP 2007-69L, G4: FLIP 04-63L, G5: Sulaimani-1, G6: Sulaimani-2, G7: FLIP 05-14L, G8: Sulaimani-3 and G9: FLIP 04-50L

Table 6. RAPD primers, the total number of amplified bands, the degree of polymorphism and size range of amplified fragments obtained in 9 lentil genotypes.

Primers	No. of amplified bands	No. of polymorphic bands	Size (bp)
NOCOD04	12	7	390-2500
OPA02	5	3	960-2450
OPA04	8	5	350-2000
OPA09	2	1	260-900
OPA10	10	8	440-1950
OPA13	7	5	330-1260
OPC05	5	3	400-1600
OPC11	8	6	370-1700
OPF03	10	7	500-1740
OPG09	4	2	325-1000
OPJ19	7	4	300-1300
OPN06	10	7	400-1200
OPN07	7	5	400-1300
OPN16	8	5	250-1280
OPP19	8	6	460-1300

OPr10	9	5	250-1200
OPR12	7	7	375-1400
OPW18	6	6	370-900
Total	133	92	
Average/primer	7.389	5.111	

Table 7. Gene diversity, PIC and positive and negative unique bands revealed among lentil genotypes using RAPD primers.

Alleles	Gene diversity	PIC	Positive unique bands	Negative unique bands
NOCOD04	0.840	0.819		1
OPA02	0.642	0.568		
OPA04	0.765	0.728		
OPA09	0.444	0.346		
OPA10	0.840	0.819	1	
OPA13	0.815	0.788		
OPC05	0.617	0.569		
OPC11	0.716	0.677	1	
OPF03	0.815	0.794	1	1
OPG09	0.519	0.485		
OPJ19	0.765	0.728		
OPN06	0.790	0.762	1	2
OPN07	0.840	0.819	2	
OPN16	0.815	0.788		
OPP19	0.790	0.762	1	1
OPr10	0.815	0.788		
OPR12	0.790	0.762		1
OPW18	0.716	0.663	1	
Mean	0.741	0.704		

Table 8. Dissimilarity matrix (based on Jaccard's coefficient) among 9 lentil genotypes based on RAPD data.

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9
G1	0.000								
G2	0.403	0.000							
G3	0.313	0.574	0.000						
G4	0.343	0.462	0.420	0.000					
G5	0.750	0.688	0.690	0.629	0.000				
G6	0.784	0.803	0.743	0.736	0.585	0.000			
G7	0.522	0.508	0.529	0.507	0.641	0.776	0.000		
G8	0.697	0.706	0.653	0.649	0.552	0.319	0.662	0.000	
G9	0.559	0.500	0.545	0.565	0.683	0.781	0.393	0.682	0.000

G1: FLIP 2006-20L, G2: FLIP 2007-53L, G3: FLIP 2007-69L, G4: FLIP 04-63L, G5: Sulaimani-1, G6: Sulaimani-2, G7: FLIP 05-14L, G8: Sulaimani-3 and G9: FLIP 04-50L.

Table 9. Analysis of molecular variance among and within populations (ICARDA and Local) of lentil based on RAPD data.

Source	DF	SS	MS	Estimated. Variance	Variation %	P-value
Among Populations	1	54.667	54.667	9.952*	40%	0.02
Within Population	7	104	14.857	14.857*	60%	0.02
Total	8	158.667		24.810	100%	

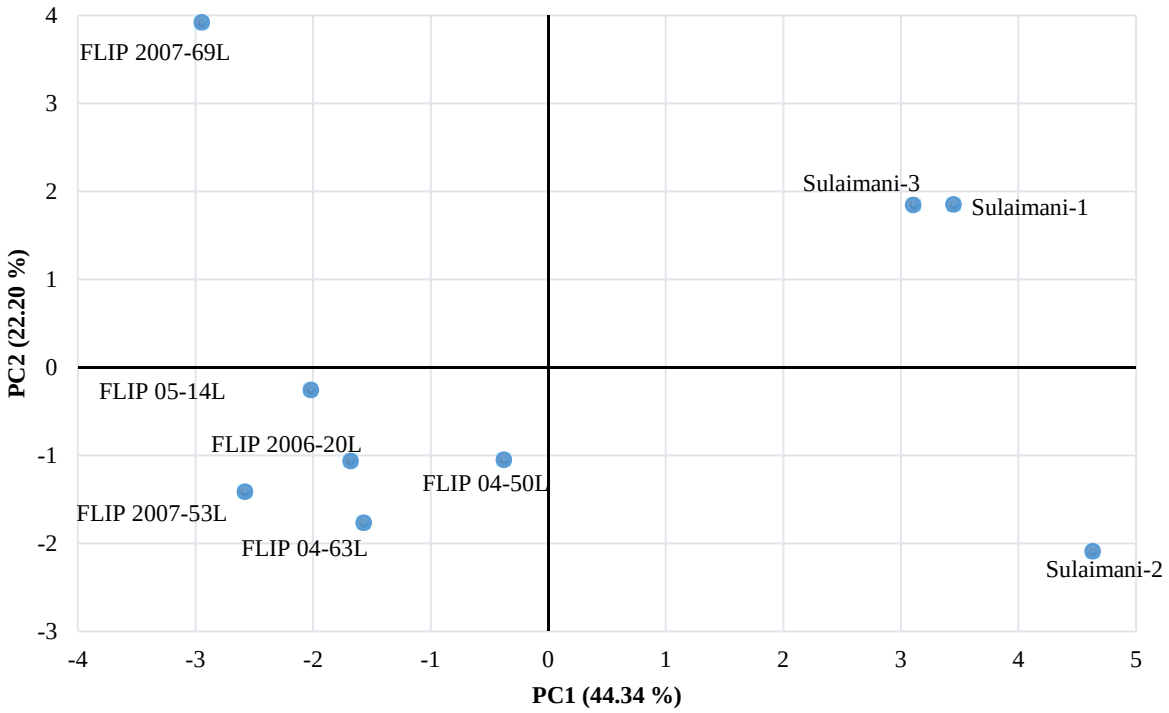

Figure 1. Principal component analyzes of nine lentil genotypes based on growth, phenological, yield component and chemical compositions data.

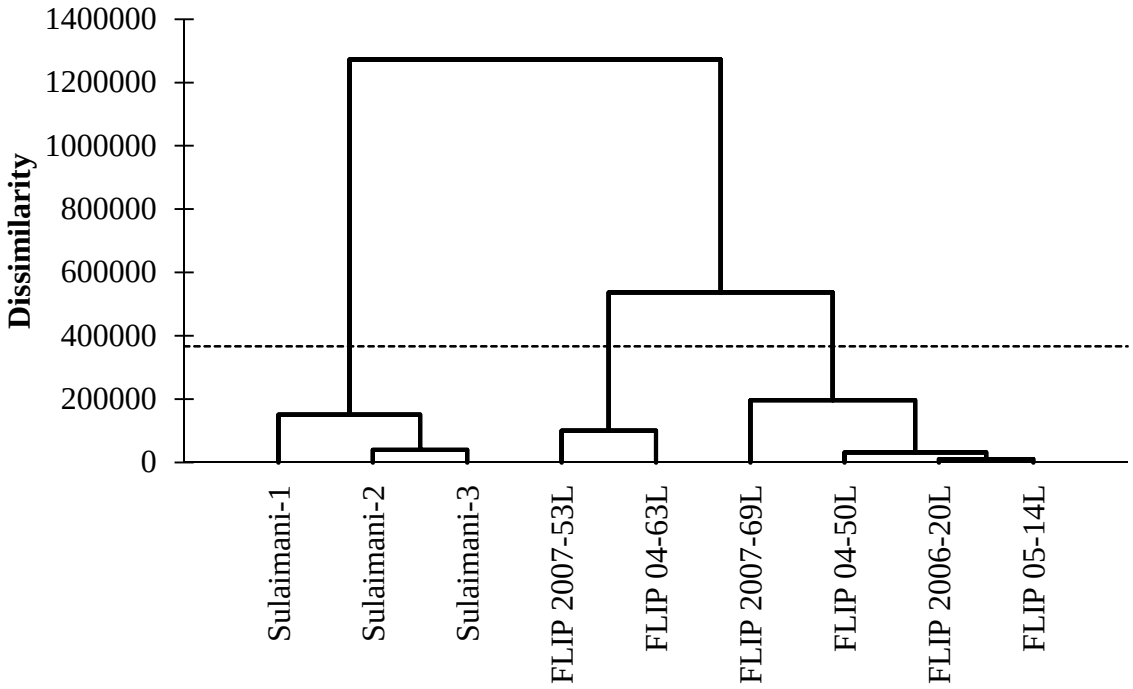

Figure 2. Dendrogram of nine lentil genotypes formed by Euclidean distance and Wards clustering methodsbased on morphological data. Dissimilarity values are indicated at the left of the dendrogram.

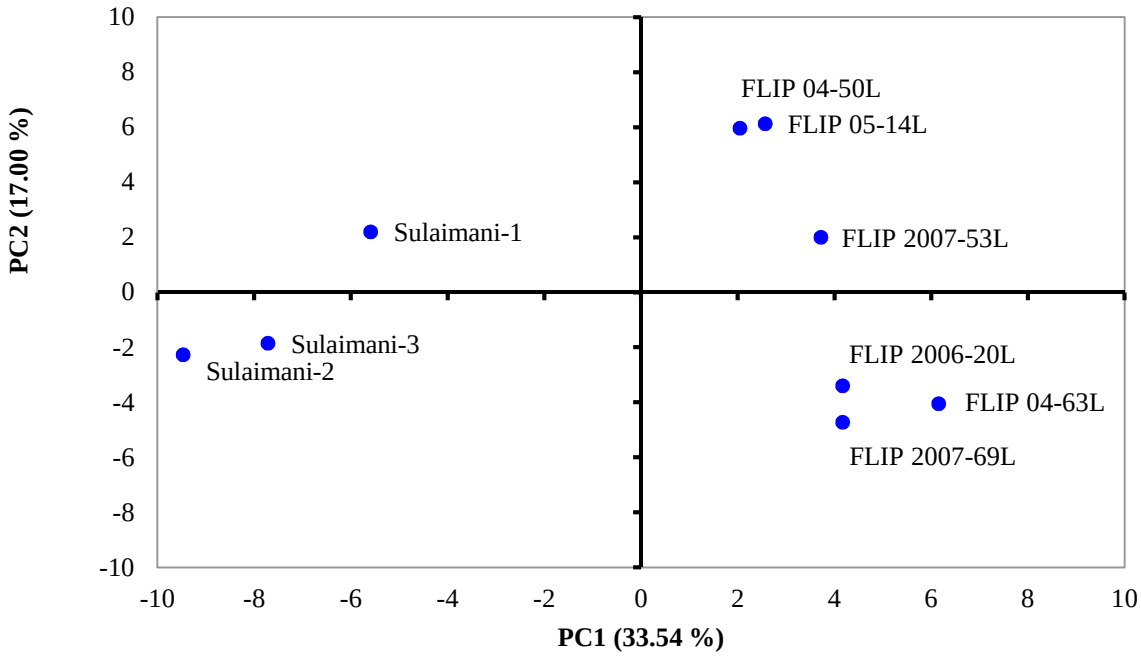

Figure 3. Principal component analyzes of first two components showing the distribution of nine lentil genotypes basing on RAPD data.

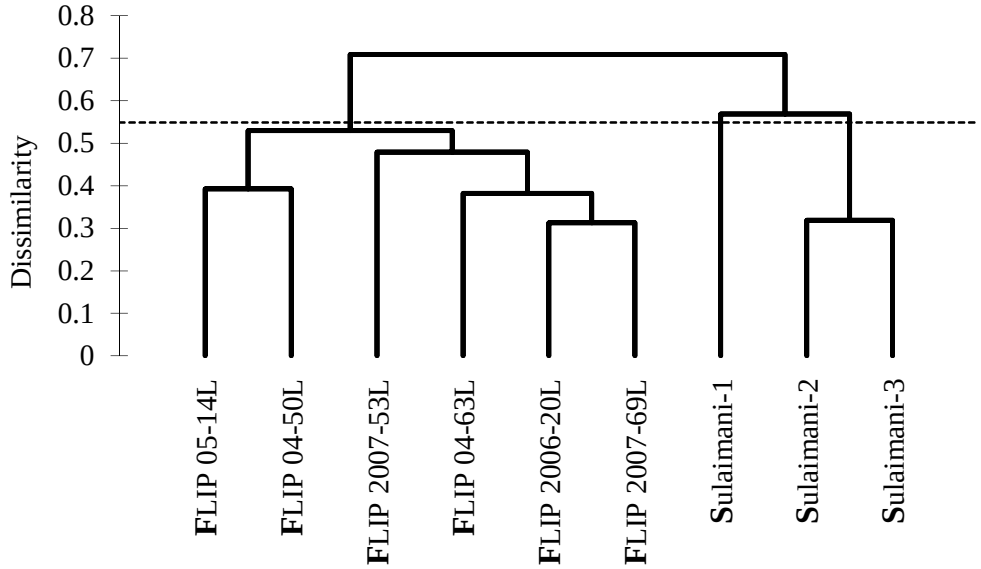

Figure 4. Dendrogram generated using Jaccard coefficient showing dissimilarity between lentil genotypes using RAPD data. Dissimilarity values are indicated at the left of the dendrogram.

Conclusion: Lentil genotypes have shown significant difference according to morphological traits, and genotype FLIP2007-69L revealed high yield potential. Results further showed that RAPD was a powerful tool for providing information about genetic diversity and polymorphism detection among the lentil genotypes.

REFERENCES

A.O.A.C. (1990). Official Method of Analysis 15th Edition, Association of Official Analytical Chemists, Washington; DC., U.S.A.

Alabboud, I., S. Lizica and G.H. Roman (2009). Assessment of genetic diversity in lentil (*Lens culinaris*Medik.) as revealed by RAPD markers. Scientific Papers, USAMV Bucharest, Series A. Vol. LII.

Afzal, M. A., M. Moynul-Haque and S. Shanmugasundaram (2004). Random amplified polymorphic DNA (RAPD) analysis of selected Mungbean (*Vignaradiata* L.) cultivars. Asian J. Plant Sci.3: 20-24.

Alghamdi, S.S., A.M. Khan, M.H. Ammar, E.H.El-Harty,H.M. Migdadi, S.M. Abd El-Khalik, A.M. Al-Shameri,M.M. Javed and Al-Faifi S.A. (2014). Phenological, nutritional and molecular diversity assessment among 35 introduced lentil (*Lens culinaris*Medik.) genotypes grown in Saudi Arabia. Int. J. Mol. Sci. 15: 277-295.

Arumuganathan, K. and E.D. Earle (1991). Nuclear DNA content of some important plant species. Plant Mol. Biol. 9: 208-218.

Ba, F.S., R.S. Pasquet and P. Gepts (2004). Genetic diversity in cowpea *(Vigna unguiculata* L. Walp.) as revealed by RAPD markers.Genet. Res. CropEvol. 51: 539-550.

Bacchi, M., M. Leone, F. Mercati,, G. Preiti, F. Sunseri and M. Monti (2010). Agronomic evaluation and genetic characterization of different accessions in lentil (*Lens culinaris*Medik.). Italian J.Agron. 4: 303-314.

Bermejo, C., I. Gatti, N. Caballero, V. Cravero, E. Martin and E. Cointry (2014). Study of diversity in a set of lentil RILs using morphological and molecular markers. Aust. J.Crop Sci. 8 (5): 689-696.

Bicer, B.T. and D. Sakar (2007). Comparison of exotic lentil lines to native cultivars for agronomic and morphologic traits. J. Agri. Sci. 13 (3): 279-284.

Chakraborty, M. and M.F. Haque(2000). Genetic variability and component analysis in lentil (*Lens culinaris* Medik.). J. Res.Birsa Agric. Univ. 12 (2): 199-204.

Clark, A. G. and C. M. C. Lanigan (1993). Prospects for estimating nucleotide divergence with RAPDs. Mol. Biol. Evol. 10: 1096–1111.

Cristóbal, M.D., V. Valentín Pando and B. Herrero (2014). Morphological characterization of lentil (*Lens Culinaris* Medik.) landraces from Castilla Y León, Spain. Pakistan J. Bot.46 (4): 1373-1380.

Doyle, J.J. and J.L. Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull.19**:**11-15.

El-Nahas, A.I., H.H. El-Shazly, S.M. Ahmed and A.A.A. Omran (2011). Molecular and biochemical markers in some lentil (*Lens culinaris* Medik.) genotypes. Ann. Agri. Sci.56 (2): 105-112.

Eriksson, L., E. Johansson, N. Kettaneh-Wold and S. Would (1999). Introduction to multi- and mega-variate data analysis using projection methods (PCA/PLS). Umetrics AB, Umeå, Sweden.

Erskine, W. and J.R. Witcombe (1984). *Lentil Germplasm Catalog*; ICARDA: Aleppo, Syria.

FAOSTAT (2015). Food and Agriculture Organization of the United Nations.

Ganjali, S., B.A. Siahsar and M. Allahdou (2012). Investigation of genetic variation of lentil lines using random amplified polymorphic DNA (RAPD) and intron-exon splice junctions (ISJ) analysis. Int. Res. J. Appl. Bas. Sci. 3 (3): 466-478.

Havey, M.J. and F.J. Muehlbauer (1989). Variabilityfor restriction fragment lengths and phylogenies in lentil. Theor. Appl. Genet.77: 839–843.

Hoque, M.E. and M.M. Hasan (2012). Molecular Diversity Analysis of Lentil (*Lens culinaris*Medik.) through RAPD Markers. Plant Tissue Cult. Biotech.22 (1): 51-58.

Jaccard, P. (1908). Nouvellesrecherches sur la distribution floral. Bull. Soc. Vaud. Sci. Nat. 44: 223–270.

Joshi, M., S.K. Verma, J.P. Singh and A. Barh (2013). Genetic diversity assessment in lentil (*lensculinaris*Medik) genotypes through ISSR marker. Bioscan 8 (4): 1529-1532.

Kaur, S., N. Cogan, L. Pembleton, M. Shinozuka, K. Savin, M. Materne and J. Forster (2011). Transcriptome sequencing of lentil based on second-generation technology permits large-scale unigene assembly and SSR marker discovery. BMC Genomics12:265.

Kumar, A.P., T. Mohapatra, T.R. Sharma, R. Bhattacharya, P.K. Dash, N.C. Gupta and K.A. Solanke (2011). Biotechnology and crop improvement. Ind. J. Agri. Sci. 81 (9): 787–800.

Lázaro, M., M. Ruiz, L. De la Rosa and I. Martín (2001). Relationships between agro/morphological characters and climatic parameters in Spanish landraces of lentil. Genet.Resour. Crop Evol. 48: 239-249.

Lichtenthaler, H. K. and C. Buschmann (2001). Chlorophylls and carotenoids: measurement and characterization by UV-VIS Spectroscopy. Current protocols in Food Analytical Chemistry. F: F4: F4.3.

Liu, J, J.P. Guan, D.X. Xu, X.Y. Zhang, J. Gu and X.X. Zong (2008). Genetic diversity and population structure in lentil (*Lens culinaris* Medik.) germplasm detected by SSR markers. Acta Agron. Sin. 34: 1901–1909.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209-220.

Mekonnen, F., F. Mekbib, S. Kumar, S. Ahmed, R.K. Chahota and T.R. Sharma (2014). Identification of molecular markers associated with rust resistance genes in lentil (*Lens culinaris*). Can. J. Plant Prot. 2: 27–36.

Mondal, M.M.A., M.A.K. Howlader, M.B. Akter and R.K. Dutta (2007). Evaluation of advanced lentil mutants in relation to morpho-physiological characters and yield. Bangladesh J. Crop Sci.18: 267-273.

Mondal, M.M.A., A.B. Puteh, M. Abdul Malek, S. Roy and M. Rafii Yusop (2013). Contribution ofmorpho-physiological traits on yield of lentil (*Lens culinaris* Medik.). Aust. J. Crop Sci. 7: 1167–1172.

Peakall, R. and P.E. Smouse(2012).GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537-2539.

Piergiovanni, A.R. (2000). The evolution of lentil (*Lens culinaris* Medik.) cultivation in Italy and its effects on the survival of autochthonous populations. Genet.Resour. CropEvol. 47:305–314.

Rana, M.K., S. Singh and K.V. Bhat (2007). RAPD, STMS and ISSR markers for genetic diversity and hybrid seed purity testing in cotton. Seed Sci. Technol. 35: 709-721.

Soller, M. and J.S. Beckmann (1983). Genetic polymorphism in varietal identification and genetic improvement.Theor. Appl. Genet. 67: 25-33.

Tayyeba, S. (2003). Geographical distribution of lentils in Pakistan as indicated by data on genetic markers.Biol. Sci. 234.

Tewari, K., H. K.Dikshit, J. Kumari, N. Jain, D. Singh and A. Singh (2015). Estimation of genetic relatedness among cultivated and wild lens based on morphological and molecular markers. Plant Gene Trait 6 (1): 1-8.

Tickoo, J.L., B. Sharma, S. K. Mishra and H.K. Dikshit. (2005). Lentil (*Lens culinaris*) in India: Present status and future perspectives. Ind. J. Agri. Sci. 75 (9): 539-67.

Toklu, F., T. Karaköy, I. Hakle, T. Bicer, A. Brandolini, B. Kilian and H. Ozkan(2009). Genetic variation among lentil (*Lens culinaris* Medik.) landraces from Southeast Turkey. Plant Breed. 128: 178–186.

Tosti, N. and V. Negri(2002). Efficiency of three PCR-based markers in assessing genetic variation among cowpea (*Vignaunguiculata* subsp. *unguiculata*) landraces. Genome 45: 268-275.

Wang, N. and J.K. Daun(2004). The Chemical Composition and Nutritive Value of Canadian Pulses, pp: 19–29. Canadian Grain Commission. (CGC), Canada.

Wang, N., D.W. Hatcher, Toews R. and E.J. Gawalko(2009). Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). Food Sci.Technol. 42: 842–848.

Xu, R.Q., N. Tomooka, D.A. Vaughan and K. Doi (2000). The *Vignaangularis complex:* genetic variation and relationships revealed by RAPD analysis, and their implications for in situ conservation and domestication. Genet.Resour. Crop Evol. 47: 123-134.

Zaccardelli, M., F. Lupo, A.R. Piergiovanni, G. Laghetti, G. Sonnante, M.G. Daminati, F. Sparvoli and L. Lioi(2012). Characterization of Italian lentil (*Lens culinaris* Medik.) germplasm by agronomic traits, biochemical and molecular markers. Genet. Resour. Crop Evol. 59: 727–738.