Brazilian Journal of Biology
ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

https://doi.org/10.1590/1519-6984.232747
Original Article

Biochemical characterization for determination of genetic distances among different indigenous chickpea (*Cicer arietinum* L.) varieties of North-West Pakistan

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Received: January 5, 2020 – Accepted: May 30, 2020
(With 5 figures)

Abstract

Genetic distances among different chickpea varieties and evaluation of their free amino acid profiles were determined on the basis of Sodium dodecyle sulphate polyacrylamide gels electrophoresis (SDS-PAGE). Total soluble proteins were resolved on 10% SDS Polyacrylamide gel. Low variability in tested varieties was observed. Dendogram based on electrophoretic data clustered the genotypes into 2 groups. The results showed that the average protein content of all the varieties was 26.01% within the range 22.8% for Thal-2006 to 34.06% Sheenghar-2000 of dry seed weight. On the basis of total protein content Bittal-98, Dasht and Sheen Ghar-2000, Karak-3 and CM-98, Paidar -91 and Fakhr-e-Thal, C-44, Balaksar and KK-1showed similar concentrations for protein contents among each other but showed variation from the rest of the varieties. Different proteins were separated on the basis of changes in their molecular weights by means of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Dasht, CM-98, and Sheen Ghar showed 100% similarity. Balaksar and Fakhr-e- Thal, KK-2 and Chattan and KC-98, KK-1 and Lawaghar were 100% similar among each other but showed variation from the rest of the accessions. The overall dendrogram showed high and low level of variation among the accessions. The concentration of free amino acids varied among the 16 chickpea varieties. A significant difference of both essential and non-essential amino acids was found among the chickpea cultivars. The total concentration of essential amino acid was recorded 40.81 g/100 g protein while non-essential was recorded 59.18343 g/100 g protein in the given cultivars. The highest concentration of essential amino acids was found in C-44 followed by KK-2, KK-1 and Fakhr E Tal while the lowest concentration was recorded in Cm-98, Paidar-91 and Sheen Ghar-2000 respectively. Cultivars TAL-2006, Chattan and Karak-3 showed maximum concentration of both essential and endogenous amino acids. In conclusion; for broadening the genetic pools in breeding programs or to search for exotic characters, for instance new disease resistance alleles, accession with low similarity coefficients (Lawaghar and Battal-98) may be utilized. Furthermore the information acquired from this study could be used to device a proficient breeding approach intended at improving nutritional as well as broadening the genetic base of this essential food crop of Pakistan.

Keywords: Chickpea, amino acid profile, SDS-PAGE, protein, genetic distances, free amino acids, genetic pool; and plant breeding program.

Caracterização bioquímica para determinação de distâncias genéticas entre diferentes variedades indígenas de grão-de-bico (*Cicer arietinum* L.) do noroeste do Paquistão

Resumo

As distâncias genéticas entre as diferentes variedades de grão-de-bico e a avaliação de seus perfis de aminoácidos livres foram determinadas com base na eletroforese em gel de poliacrilamida com dodecil sulfato de sódio (SDS-PAGE). As proteínas solúveis totais foram resolvidas em SDS-PAGE a 10%. Foi observada baixa variabilidade nas variedades testadas. O dendrograma fundamentado em dados eletroforéticos agrupou os genótipos em dois grupos. Os resultados mostraram que o teor médio de proteínas de todas as variedades foi de 26,01%, na faixa de 22,8% para Thal-2006 a 34,06% para Sheenghar-2000 do peso de sementes secas. Com base no conteúdo total de proteínas, Bittal-98, Dasht, Sheen Ghar-2000, Karak-3, CM-98, Paidar-91, Fakhr-e-Thal, C-44, Balaksar e KK-1 apresentaram concentrações semelhantes para o conteúdo de proteínas entre si, mas tiveram variação quanto ao restante das variedades.

Diferentes proteínas foram separadas com base nas alterações de seus pesos moleculares por meio de eletroforese em gel de poliacrilamida com dodecil sulfato de sódio (SDS-PAGE). Dasht, CM-98 e Sheen Ghar mostraram 100% de similaridade. Balaksar, Fakhr-e-Thal, KK-2, Chattan e KC-98, KK-1 e Lawaghar foram 100% semelhantes entre si, mas apresentaram variação em relação ao restante dos acessos. O dendrograma geral mostrou alto e baixo nível de variação entre os acessos. A concentração de aminoácidos livres variou entre as 16 variedades de grão-de-bico. Foi encontrada uma diferença significativa entre os aminoácidos essenciais e não essenciais nas cultivares de grão-de-bico. A concentração total de aminoácidos essenciais foi registrada em 40,81 g / 100 g de proteína, enquanto a não essencial foi registrada em 59,18343 g / 100 g de proteína nas cultivares. A maior concentração de aminoácidos essenciais foi encontrada em C-44, seguida de KK-2, KK-1 e Fakhr-e-Thal, enquanto a menor concentração foi registrada em CM-98, Paidar-91 e Sheen Ghar-2000. As cultivares TAL-2006, Chattan e Karak-3 apresentaram concentração máxima de aminoácidos essenciais e endógenos. Em conclusão, para ampliar os pools genéticos em programas de melhoramento ou procurar caracteres exóticos, por exemplo, novos alelos de resistência a doenças, pode ser utilizada a adesão com baixos coeficientes de similaridade (Lawaghar e Battal-98). Além disso, as informações adquiridas neste estudo poderiam ser usadas para criar uma abordagem de criação eficiente, com o objetivo de melhorar a nutrição e ampliar a base genética dessa cultura alimentar essencial do Paquistão.

Palavras-chave: Grão-de-bico, perfil de aminoácidos, SDS-PAGE, proteína, Distâncias genéticas, aminoácidos livres, pool genético; e programa de melhoramento de plantas.

1. Introduction

Chickpea (CicerarietinumL.) is an annual grain legume, self-pollinated, diploid (2n = 16) crop. It is comprised of a significant source of dietary proteins. It is the third most important legume cultivated crop of the Indian sub-continent, North Africa and West Asia (Bharadwaj et al., 2010; FAOSTAT, 2011). Chickpea is also the most important nutritive seed crop with high protein content varying between 20 to 26%. It is also valued as a good source of zinc, folate, phosphorus, iron and certain water soluble vitamins. Chickpea, as a high dietary fiber and carbohydrate food contributes a significant food source for persons with insulin sensitivity or diabetes (Singh and Jambunathan (1982); Jukanti et al., 2012). Its seed contains 3% fiber, 4.8 to 5.5% oil, 3% ash, 0.2% calcium, and 0.3% phosphorus, 60 to 66% carbohydrate content, while a small amount of (1 to 6%) lipids., while a small amount of (1 to 6%) lipids depending on different varieties (Hulse, 1991; Huisman and Poel, 1994). Chickpea is a good source of carbohydrates and protein which constitute about 80% of the total dry seed mass (Chibbar et al., 2010; Dhawan et al., 1991; Geervani, 1991). There are two kinds of gram crop, namely Desi and Kabuli in Pakistan. Desi (microsperma) types have a colored and thick seed coat, pink flowers and anthocyanin pigmentation on stems. The kabuli (macrosperma) types have white or beige-colored seeds with a ram's head shape, white flowers and thin seed coat and smooth seed surface. They lack anthocyanin pigmentation on stem (Moreno and Cubero, 1978). The weight of the seeds commonly ranges between 0.1 to 0.3g and 0.2 to 0.6g in desi and kabuli types, respectively (Frimpong et al., 2009). In Africa and Asia the Desi type contributes about 80-85% of the total chickpea area while in Europe and America the Kabuli types are largely grown (Pande and Kishore, 2005). Simon and Muehlbauer (1997) reported that Chickpea (gram) crop is usually cultivated as a single crop or combined with barley, linseed, mustard, pea, sweet potato, wheat, or sorghum, etc. Knowing genetic diversity not only helps in sorting of populations for genome mapping experiments but also accounts an important tool in gene-bank management and breeding experiments like tagging of germplasm, identification and/or elimination of duplicates in the gene stock and establishment of core collections (Kaga et al. 1996). The advancements in germplasm characterization using biochemical fingerprinting has got special attributes due to its increased use in crop improvement and the selection of desirable genotypes for breeding crops. The taxonomic and evolutionary problems of several crop plants have been resolved by using genetic markers and protein profiling (Boutler et al., 1966; Gepts et al., 1988; Gepts and Bliss, 1988; Ladizinsky and Hymowitz, 1979; Murphy et al., 1990; Khan, 1990; Nakajima, 1994; Najma et al., 2005; Rao et al., 1992; Das and Mukarjee, 1995; Ghafoor et al., 2002; Javid et al., 2004). Significant studies have been reported regarding the genetic diversity of seed storage proteins for many crops; Lima bean (Lioi et al., 1999), *Phaseolus vulgaris* (Ferreira et al., 2000) and *Cicerarietinum* (Ghafoor et al., 2003). Based on SDS-PAGE data, Ahmad and Slinkard (1992) studied phylogenetic relationship among Cicer species and suggested that *Cicerreticulatumis* would be the wild progenitor of cultivated chickpea. The gene homology being the basic criterion of phylogenetic relationship cannot in many cases be measured directly because of reproductive barriers between species. Therefore seed storage protein analysis is not only regarded as a useful tool in identification and characterization of diversity in crop varieties, cultivars and their wild varieties but also helps to elaborate genetic transgression and phylogenetic relationship of the accessions (Ahmad et al., 2007; Hussain et al., 2010). It is also reported that variations in protein bands show the relationship among the collection from various geographical regions (Valizade (2001); Satija et al., 2002; Ghafoor et al., 2003; Asghar et al., 2003). The present study was undertaken to estimate the genetic distances among indigenous chickpea varieties of North-West Pakistan based on their biochemical characteristics, amino acids profiling, protein content and band profiling.

2. Materials and methods

2.1. Plant material

The different indigenous chickpea varieties/genotypes widely cultivated in North-West Pakistan; used during the present study were C-44, Karak-3, Bittal-98, Dacht, Sheenghar-2000, Paidar-91, CM-98, Balaksar, Pb-91, Thal-2006, Chattan, KC-98, Lawaghar, KK-1, Fakhr-e-Thal and KK-2).

2.2. Preparation of protein samples

Total soluble proteins were extracted by grounding the seeds in 50 mM phosphate buffer (pH 7.8) and centrifuged at 14,000 rpm for 10 minutes. The supernatant was separated and used for protein concentration and profiling.

2.3. Protein Quantification

CBB-dye binding assay as described by Bradford (1976) was used to measure the protein concentration.

2.4. Protein profiling using SDS-PAGE:

Protein profiling of samples was performed using Sodium dodecyle sulphate polyacrylamide gels as described by Laemmli (1970).

2.5. Amino Acid profiling

The dried seeds were ground with the help of mortar and pestle. Extraction of amino acids was carried out according to the method described by Mansfield and Baerlocher (2005). A total of 0.3 g of seeds powder was taken in falcon tubes and 5 ml HCl (0.1%) were added. The sample was homogenized through vortexing for 5 minutes. After vortexing the sample was centrifuged at 3500 rpm for 15 minutes and the supernatant was collected in separate tubes and labeled. The amino acid were analyzed using High Performance Liquid Chromatography (HPLC). The system was equipped with UV 338nm detector, column with C 18, 2.5 x 200mm, 5µm column. The mobile phase constituted 1:2:2 (100mM sodium sulphate, pH 7.2; acetonitrile; methanol (v/v/v) with a flow rate of 0.45 ml/minute. The operating temperature was set at 40°C.

2.6. Statistical analysis

The data was analyzed using SPSS to the general linear model (GLM) procedure of statistical analysis system. The means were compared by LSD and all pair wise comparison test. Significance was found at ($P<0.05$).

3. Results

During the current study, an effort was made to estimate the genetic distances among different indigenous chickpea varieties of North-West Pakistan based on biochemical characteristics, amino acid profiling, protein content and band profiling.

3.1. Proteins Quantifications:

Total soluble proteins in different chickpea seeds ranged from 228.2 mg/g (22.8%) to 340.6 mg/g (34.0%) of dry seed weight (Supplementary Material Table 1). The average protein content of all the varieties was 26.01%. Highest percentage for protein content was recorded for Sheen Ghar-2000 while the lowest protein content was observed in Thal-2006. On the basis of total protein content Bittal-98, Dasht and Sheen Ghar-2000, Karak-3 and CM-98, Paidar -91 and Fakhr-e-Thal, C-44, Balaksar and KK-1showed similar concentrations for protein contents among each other but showed variation from the rest of the varieties (Figure 1).

3.2. SDS-PAGE based Genetic Diversity

SDS-PAGE was utilized to estimate the extent of genetic diversity existing among the germplasm of chickpea. Different proteins were separated on the basis of change in their molecular weights of all the tested varieties by means of SDS-PAGE. The binary data matrix of all the genotypes was computed for the formation of phylogenetic tree (dendrogram) using UPGMA. Total of 13 bands were observed. The cluster analysis was performed for total banding pattern.

3.3. Cluster analysis on the basis of SDS-PAGE

Cluster analysis of chickpea seed storage proteins was performed on the results of SDS-PAGE (Figure 2 and 3). The software "popgene" was used to calculate the genetic diversity among the given varieties. The results of cluster analysis are presented as phylogenetic tree (dendogram) in Figure 4 on the basis of linkage distance by using "Un-weighted pair group method with arithmetic means" (UPGMA) (Nei and Li, 1979).

In cluster analysis for whole polypeptide bands, the whole samples were divided into two groups or lineages, lineage 1 (L1) and lineage 2 (L2), at a linage distance of 0.25 (Figure 4). These lineages were further divided into clusters at linkage distance 0.15, forming five clusters (C1 – C5). Lineage 1 consists of two clusters while lineage 2 consists of 3 clusters. Among the linage

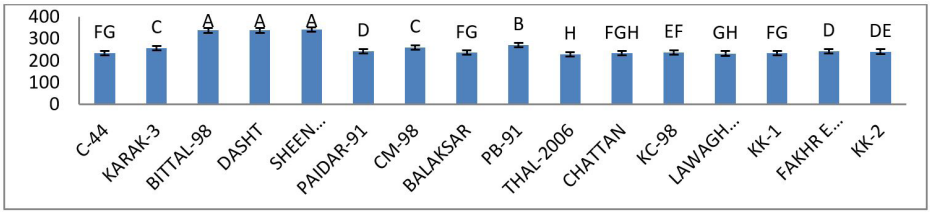

Figure 1. Mean concentration values (mg/g) for total seed storage proteins of sixteen different chickpea varieties.

1, cluster 1 (C1) consists of a single accession, while cluster 2 (C2) contains two accessions. Similarly in linage 2, cluster 3 (C3) consists of a single accession while cluster 4 (C4) and clusters 5 (C5) consists of two accessions each. Cluster 2 and 4 were further divided into sub-clusters at linkage distance of 0.06. The percentage of each cluster in total population (16 genotypes) is given in Table 1.

Table 1. Cluster analysis based on SDS-PAGE (1-13 bands).

Lineage	Cluster	Percentage in total population	Genotype
L1	C1	6.25%	Bittal-98
	C2	25%	Paidar-91, Dasht, CM-98, Sheenghar-2000,
L2	C3	12.5%	Balaksar, Fakhr-e-Thal
	C4	25%	Karak-3, C-44, Pb-91, Thal-2006
	C5	31.25%	KK-2, Chattan, KC-98, KK-1, Lawaghar

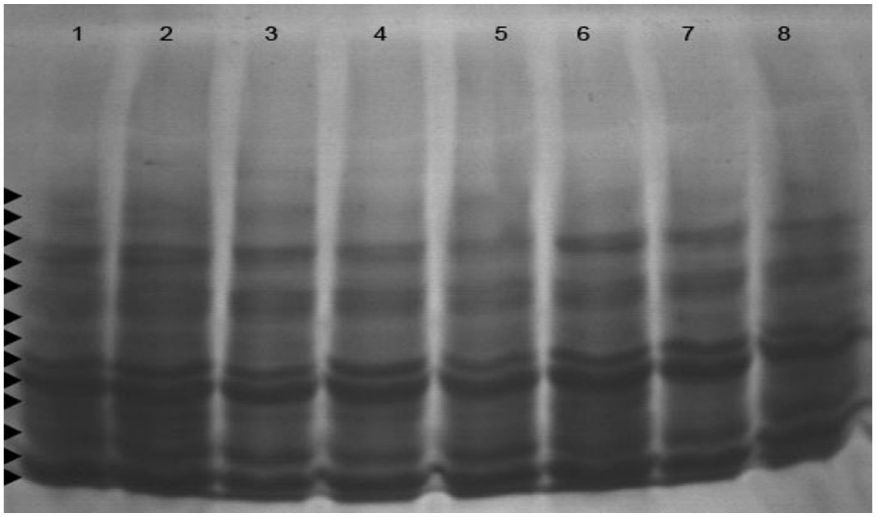

Figure 2. Electrophorogram of 10% Polyacrylamide gel banding pattern showing diversity in total seed proteins of Chickpea (1=C-44, 2=Karak-3,3= Bittal-98, 4=Dasht, 5=Sheenghar-2000, 6=Paidar-91,7=CM-98, 8=Balaksar).

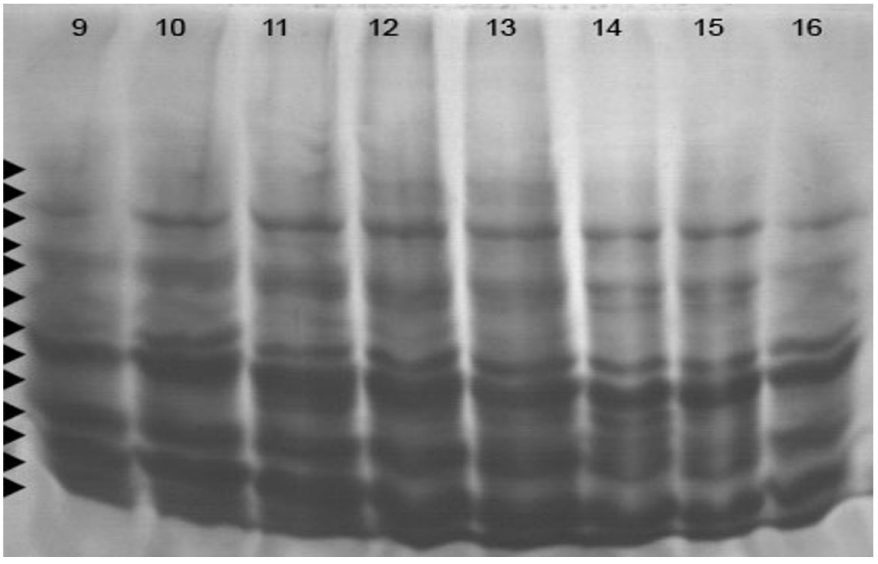

Figure 3. Electrophorogram of 10% Polyacrylamide gel banding pattern showing diversity in total seed storage proteins of Chickpea (9=Pb-91, 10=Thal-2006, 11=Chattan, 12=KC-98, 13=Lawaghar, 14=KK-1, 15=Fakhr-e-Thal, 16=KK-2).

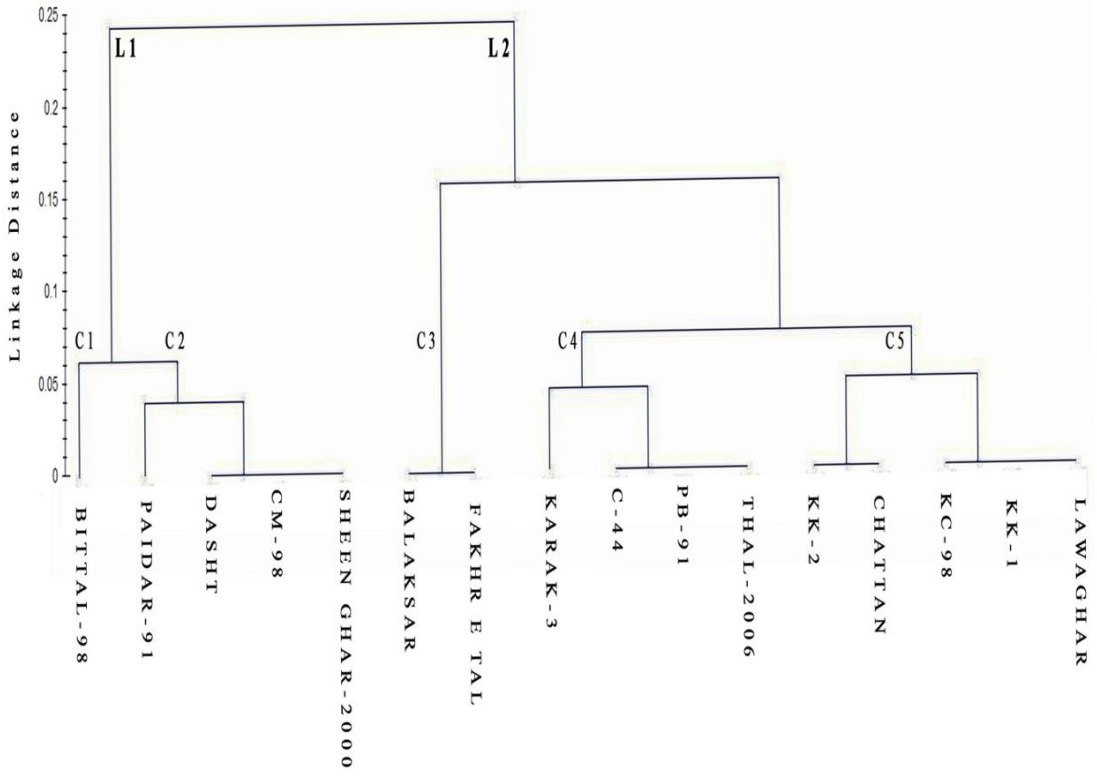

Figure 4. Dendogram constructed for 16 different chickpea (*Cicerarietinum*L.) varieties using SDS PAGE homologous variety sets. Lineage 1 (L1) (Bittal-98, Paidar-91, Dasht, CM-98, Sheen ghar-2000) Lineage 2 (L2) (Balaksar, Fakhr-e-Thal, Karak -3, C-44, PB-91, Thal-2006, KK-2, Chattan, KC-98, KK-1, Lawaghar.

In lineage 1 Bittal-98 showed highest variations at LD = 0.06, In lineage 2 Balaksar and Fakhr-e-Thal showed variation from the rest of the accessions in the same clusters at LD= 0.16 while in cluster 4 Karrak-3 showed variation at LD = 0.06. In lineage 1 accession Dasht, CM-98, and Sheen Ghar showed 100% similarity at LD = 0.03 and could not be differentiated among each other on the basis of total protein bands.

In lineage 2 at LD = 0.16 accession Balaksar and Fakhr-e-Thal at cluster 3, at LD = 0.08 accession C-44, PB-91 and Thal-2006 at cluster 4 and at LD= 0.08 accession KK-2 and Chattan at cluster 5 and KC-98, KK-1 and Lawaghar at cluster 5 were 100% similar among each other but showed variation from the rest of the accessions. Therefore, they were grouped in each cluster. The overall dendogram showed high and low level of variation among the accessions (Figure 4). The average variation observed was 30%. Accession line; Balaksar showed maximum variation whereas Karak-3 showed the lowest variation. Sheen Ghar-2000, Thal-2006 and CM-98 did not show any variation (Table 2).

4. Amino Acids profiling

4.1. Amino Acids profiling of Chickpea

Amino acids are significant component of the food and play an important role to indicate its nutritional value. The concentration of free amino acids varied among the 16 chickpea cultivars. Highest concentration for alanine was recorded in Thal-2006, Arginine in CM-98, Aspartate, Isoleucine, tyrosine and Histidine in Lawaghar, Glutamate in Chattan, glycine, Leucine and lysine in KK-2, Methionine and valine in C44, Phenylalanine in Pb-91, Proline and Threonine in Fakhr-e-Thal and serine in Dasht. The lowest concentration of alanine was observed in Karak-3 and KC-98, Glycine in C-44, Arginine and lysine in Fakhr-e-Thal, Aspartate and Glutamate in Thal-2006, Histidine in Paidar, Leucine in Paidar and CM-98, and valine in Pb-91, Methionine in Sheenghar-2000, phenylalanine in CM-98 and KC-98, Proline in CM-98, serine in CM-98 and Fakhr-e-Thal, Threonine in KC-98 and Tyrosine in Dasht (Table 3) . Furthermore the total concentration of essential amino acid was recorded 40.81 g/100 g protein while non-essential was recorded 59.18343 g/100 g protein in the given cultivars. A significant difference of both essential and non-essential amino acids was found among the chickpea cultivars. The highest concentration of essential amino acids was found in C-44 followed by KK-2, KK-1 and FAKHR E TAL while the lowest concentration was recorded in CM-98, PAIDAR-91 and SHEEN GHAR-2000 respectively (Figure. 5).

Table 2. Dissimilarity matrix for protein banding profile data based on 1-13 polypeptide bands.

	C-44	KARAK-3	BITTAL-98	DASHT	SHEEN GHAR-2000	PAIDAR-91	CM-98	BALAKSAR	PB-91	THAL-2006	CHATTAN	KC-98	LAWAGHAR	KK-1	FAKHR E TAL	KK-2	AVERAGE
C-44	0.00																0.00
KARAK-3	0.08																0.08
BITTAL-98	0.46	0.42															0.44
DASHT	0.54	0.50	0.14														0.39
SHEEN GHAR-2000	0.54	0.50	0.14	0.00													0.30
PAIDAR-91	0.46	0.42	0.25	0.14	0.14												0.28
CM-98	0.54	0.50	0.14	0.00	0.00	0.14											0.22
BALAKSAR	0.23	0.31	0.69	0.67	0.67	0.58	0.67										0.55
PB-91	0.00	0.08	0.46	0.54	0.54	0.46	0.54	0.23									0.36
THAL-2006	0.00	0.08	0.46	0.54	0.54	0.46	0.54	0.23	0.00								0.32
CHATTAN	0.15	0.23	0.36	0.46	0.46	0.36	0.46	0.39	0.15	0.15							0.32
KC-98	0.08	0.15	0.42	0.50	0.50	0.42	0.50	0.31	0.08	0.08	0.08						0.28
LAWAGHAR	0.15	0.23	0.50	0.46	0.46	0.36	0.46	0.25	0.15	0.15	0.17	0.08					0.29
KK-1	0.23	0.31	0.46	0.40	0.40	0.30	0.40	0.33	0.23	0.23	0.25	0.17	0.09				0.29
FAKHR E TAL	0.31	0.39	0.40	0.33	0.33	0.40	0.33	0.42	0.31	0.31	0.33	0.25	0.18	0.10			0.31
KK-2	0.31	0.39	0.55	0.50	0.50	0.40	0.50	0.27	0.31	0.31	0.33	0.39	0.33	0.27	0.36	0.00	0.38

Table 3. Mean concentration values for Amino acid (g/100g sample) of total seed storage proteins of sixteen different chickpea varieties.

	C-44	KARAK-3	BITTAL-98	DASHT	SHEEN GHAR-2000	PAIDAR-91	CM-98	BALAKSAR	PB-91	TAL-2006	CHATTAN	KC-98	LAWAGHAR	KK-1	FAKHR E TAL	KK-2
ASP	0.61	0.99	0.54	0.80	1.05	0.66	0.38	0.84	0.55	0.26	1.11	0.36	1.13	1.11	0.97	0.93
THR	0.70	1.33	0.34	0.97	1.19	1.10	0.64	1.25	1.02	0.44	1.00	0.17	0.87	1.41	2.26	1.65
SCR	0.45	0.59	0.56	2.95	0.78	0.71	0.36	0.89	0.86	0.62	0.52	0.67	0.41	0.61	0.36	0.65
GLU	2.72	3.29	2.30	0.00	3.07	2.61	1.70	3.41	3.34	1.01	3.49	2.79	3.06	3.04	0.00	3.25
PRO	0.45	0.84	0.71	0.93	0.62	0.72	0.37	0.80	0.88	0.40	0.82	0.58	0.78	0.77	2.59	0.85
GLY	0.23	0.84	0.63	0.61	0.53	0.71	0.37	0.69	0.71	0.80	0.94	0.70	1.03	1.15	0.78	1.40
ALA	0.00	0.40	1.64	1.98	1.24	0.94	2.95	1.16	1.88	3.45	1.33	0.40	0.77	0.90	0.96	1.12
VAL	4.18	2.45	2.12	2.13	1.33	0.63	0.89	1.40	0.29	2.68	1.87	0.77	0.00	1.16	0.84	1.14
MET	3.26	1.05	0.00	0.40	0.00	0.32	0.32	0.50	0.22	0.65	0.24	1.42	1.26	1.84	1.22	0.23
ILE	0.70	0.13	1.47	0.11	0.12	0.00	0.49	0.50	1.45	0.83	1.21	0.14	2.49	0.33	2.02	0.62
LEU	0.17	0.38	0.24	0.25	0.55	0.15	0.15	0.57	0.45	0.69	0.55	0.88	0.24	1.33	0.42	1.39
TYR	1.14	0.13	0.07	0.66	0.55	0.32	0.00	0.54	0.77	0.00	0.00	0.39	1.60	0.22	1.31	0.34
PHE	0.23	0.30	0.23	0.38	0.40	0.33	0.22	0.42	0.51	0.50	0.00	0.22	0.33	0.24	0.29	0.30
HIS	0.59	1.18	1.26	0.63	0.53	0.33	1.02	0.66	0.62	2.59	1.13	0.48	2.71	0.36	0.54	0.39
LYS	0.34	0.93	1.07	0.81	0.60	0.54	0.49	0.72	1.17	0.44	1.57	1.33	0.00	1.52	0.32	1.68
NH3	0.61	0.08	0.16	0.70	0.92	0.55	0.13	0.84	1.06	1.28	0.42	0.21	0.00	0.54	1.70	0.43
ARG	0.28	1.77	3.32	2.38	3.20	6.06	6.20	1.49	0.90	0.00	0.44	5.15	0.00	0.12	0.09	0.30

5. Discussion

Some of the significant factors that determine the level of genetic variability are extent of distribution; areas sampled and plant characteristics such as mode of reproduction, breeding behavior and generation time. Total soluble proteins of 16 different chickpea varieties extracted and quantified showed that the average protein content of all the varieties was 26.01% within the range of 22.8% for Thal-2006 to 34.06% Sheenghar-2000 of dry seed weight. Our findings are complementary with the results of Milan-Carillo et al. (2000) who have recorded mean value of 22.5% protein for desi chickpea genotypes. Protein content are also in concordance with Jambunathan and Singh (1981) who analyzed 8 desi and 7 kabuli chickpea cultivars and observed higher crude protein for kabuli types (241 g/kg) than desi type (217g/kg). Difference in crude protein content has been reported to depend on geographical origin of seed in addition to genetic differences but the role of location and season in the genotypic expression of protein content is low. Indian subcontinent has a very diverse Germplasm and its diversity includes the total diversity expressed by the exotic accession. One of the most extensively used and reliable techniques to separate and differentiate proteins is SDS-PAGE (Javed et al., 2004; Iqbal et al., 2005). It is used to separate different proteins on the basis of changes in their molecular weights. A powerful tool for population genetics is protein electrophoresis (Parker et al., 1998). In the present study the 16 different chickpea varieties showed both high and low levels of genetic variations during cluster analysis which is in agreement with Hameed et al. (2009), who reported the same results for seed storage protein of Kabuli Chickpea genotypes. The whole polypeptide bands were divided into two groups or lineages, Lineage 1 and lineage 2, at a linkage distance of 0.25. These lineages were further divided into clusters at lineage distance of 0.15, forming five clusters (C1-C5). Lineage 1 contains 2 clusters while lineage 2 contains 3 clusters. Among the linage 1, cluster 1 (C1) consists of a single accession, while cluster 2 (C2) contains two accessions. Similarly in linage 2, cluster 3 (C3) consists of a single accession while cluster 4 (C4) and clusters 5 (C5) consists of two accessions each. Cluster 2 and 4 were further divided into sub-clusters at linkage distance of 0.06. The percentage of each cluster in total population (16 genotypes) is given in Table 1. In lineage 1 Bittal-98 showed highest variations at LD = 0.06. In lineage 2 Balaksar and Fakhr-e-Thal showed variation from the rest of the accessions in the same clusters at LD= 0.16 while in cluster 4 Karrak-3 showed variation at LD = 0.06 In lineage 1 accession Dasht, CM-98, and Sheen Ghar showed 100% similarity at LD = 0.03 and could not be differentiated among each other on the basis of total protein bands. In lineage 2 at LD = 0.16 accession Balaksar and Fakhr-e- Thal at cluster 3, at LD = 0.08 accession C-44, PB-91 and Thal-2006 at cluster 4 and at LD= 0.08 accession KK-2 and Chattan at cluster 5 and KC-98, KK-1 and Lawaghar at cluster 5 were 100% similar among each other but showed variation from the rest of the accessions. Therefore, they were grouped in each cluster. The overall dendogram showed high and low level of variation among the accessions (Figure 4). The average variation observed was 30% (Table 2) showing a significant variation earlier examined in various studies. Hameed et al., 2009 studied eight Kabuli Chickpea genotypes and observed total variation of 20%. Similarly Ahmad et al. (2012) found 37% variation based on molecular characterization of Chickpea germplasm. A low level of intra specific variation was studied among the chickpea accessions, which confirms the reports of Thakare et al. (1987), Mehrani (2002) and Ghafoor et al. (2003). This low level of variation signifies a risk to the genotypes examined in the study therefore variation must be high for excellent yield, disease resistance and other environmental susceptibility, reducing the chances of harmful effect of environment and other factors. SDS-PAGE results confirmed that the method provides a tool for reliable germplasm characterization based on genetic dissimilarities in seed storage proteins composition in chickpea. Our results did not confirm the findings of Nisar et al. (2007) who studied considerable intra specific variation in local and exotic chickpea germplasm. Large amounts of proteins are stored in Legume seeds during their development. They do not play any role in the development of cotyledonary tissue therefore they are accumulated in storage vacuoles or protein bodies. During seed maturation, the cotyledonary parenchyma cells survive desiccation and go through proteolysis at germination, thus supply free amino acids, as well as ammonia and carbon skeletons to the developing seedlings. These seed proteins are called storage proteins (Casey et al., 1986). Several comparatively minor proteins, including protease and amylase inhibitors, lectins, lipoxygenase and defense proteins are found in Legume seeds which are important to the nutritional/functional quality of the seed (Murray, 1979). The potential quality of a protein food is presented by its Amino acid composition therefore their bioavailability is critical for the supply of amino acids in the diet (Sarwar and Peace, 1986). A higher amount of essential amino acid is found in all chickpea varieties (WHO, 1973; FAO, 1973). Amino acid content normally shows the nutritive value of a protein source (Bodwell et al., 1980) and is broadly used for screening prospective protein foods Zia-Ul-Haq et al. (2007). The concentration of free amino acids varied among the 16 chickpea varieties. Highest concentration for alanine was recorded in Thal-2006, Arginine in CM-98, Aspartate, Isoleucine, tyrosine and Histidine in Lawaghar, Glutamate in Chattan, glycine, Leucine and lysine in KK-2, Methionine and valine in C44, Phenylalanine in Pb-91, Proline and Threonine in Fakhr-e-Thal and serine in Dasht. The lowest concentration of alanine was observed in Karak-3 and KC-98, Glycine in C-44, Arginine and lysine in Fakhr-e-Thal, Aspartate and Glutamate in Thal-2006, Histidine in Paidar, Leucine in Paidar and CM-98, and valine in Pb-91, Methionine in Sheenghar-2000, phenylalanine in CM-98 and KC-98, Proline in CM-98, serine in CM-98 and Fakhr-e-Thal, Threonine in KC-98 and Tyrosine in Dasht. Furthermore

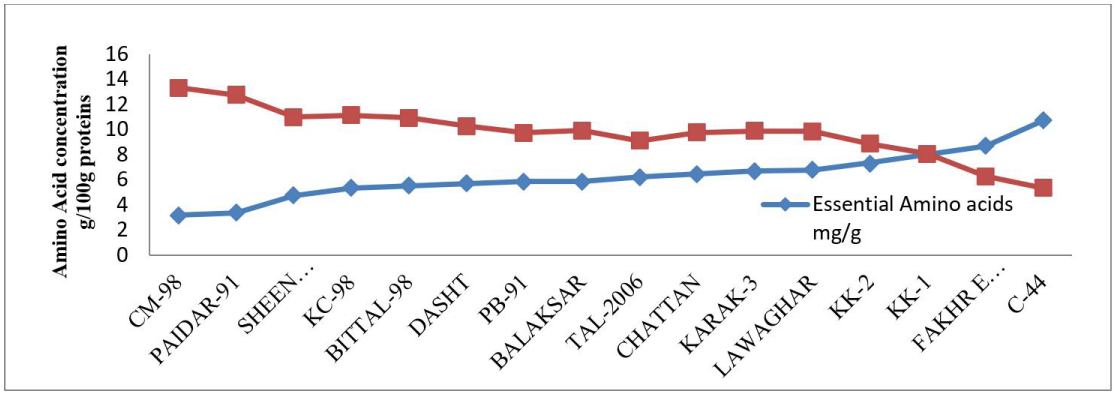

Figure 5. Concentraion of both essential and non-essential amino acids in 16 different chickpea cultivars.

the total concentration of essential amino acid was recorded 40.81 g/100 g protein while non-essential was recorded 59.18343 g/100 g protein in the given cultivars. Our results are similar with findings of Alajaji and El-Adawy (2006) and Zia-Ul-Haq et al. (2007) who studied indigenous varieties in Punjab Pakistan. Cultivars C-44, KK-2, KK-1 and FAKHR E TAL showed a significant variation of essential amino acids from the rest of the cultivars; while cultivars TAL-2006, CHATTAN and KARAK-3 showed maximum concentration of both essential and endogenous amino acids (Figure 5).

6. Conclusion

To broaden the genetic pools in breeding programs or to search for exotic characters, for instance new disease resistance alleles, accession with low similarity coefficients (Lawaghar and Battal-98) may be utilized. Conversely it is very complicated to establish the genetic similarities based on seed storage protein profile to reveal genetic diversity, therefore DNA based genetic markers such as SSR, RAPD should be used to further investigate related varieties of chickpea to determine its genetic identity. The information acquired from this study could be used to device a proficient breeding approach intended at improving nutritional as well as broadening the genetic base of this essential food crop of Pakistan. Since the information attained reveals the potential worth of Chickpea germplasm collections therefore efforts are being made to expand the data base by characterizing the remaining germplasm.

Acknowledgements

We are thankful to NIFA, Tarnab and Pulse Research Institute Karak; Pakistan for providing different varieties/ genotypes of chickpea.

References

AHMAD, F. and SLINKARD, A.E., 1992. Genetic relationships in the genus *Cicer* L., as revealed by polyacrylamide gel electrophoresis of seed storage proteins. *Theoretical and Applied Genetics*, vol. 84, no. 5-6, pp. 688-692. http://dx.doi.org/10.1007/BF00224169. PMid:24201358.

AHMAD, H., NISAR, M., GHAFOOR, A., KHAN, M.R., QURESHIAND, A.S. and ALI, H., 2007. Genetic diversity and geographic relationship among local and exotic chickpea germplasm. *Pakistan Journal of Botany*, vol. 39, no. 5, pp. 1575-1581.

AHMAD, Z., MUMTAZ, A.S., NISAR, M. and KHAN, N., 2012. Diversity analysis of chickpea (*Cicer arietinum* L.) germplasm and its implications for conservation and crop breeding. *Agricultural Sciences*, vol. 3, no. 5, pp. 723-731. http://dx.doi.org/10.4236/as.2012.35087.

ALAJAJI, S.A. and EL-ADAWY, T.A., 2006. Nutritional composition of chickpea (*Cicer arietinum* L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis*, vol. 19, no. 8, pp. 806-812. http://dx.doi.org/10.1016/j.jfca.2006.03.015.

ASGHAR, R., SIDDIQUE, T. and AFZAL, M., 2003. Inter and intra-specific variation in SDS-PAGE electrophoregrams of total seed protein in chickpea (*Cicer arietinum* L.) germplasm. *Pakistan Journal of Biological Sciences*, vol. 6, no. 24, pp. 1991-1995. http://dx.doi.org/10.3923/pjbs.2003.1991.1995.

BHARADWAJ, C., CHAUHAN, S.K., RAJGURU, G., SRIVASTAVA, R., SATYAVATHI, C.T., YADAV, S., RIZVI, A.H., KUMAR, J. and SOLANKI, R.K., 2010. Diversity analysis of Chickpea (*Cicer arietinum*) cultivars using STMs Markers. *Indian Journal of Agricultural Sciences*, vol. 80, no. 11, pp. 947-951.

BODWELL, C.E., SATTERLEE, L.D. and HACKLER, L.R., 1980. Protein digestibility of the same protein preparations by human and rat assays and by in vitro enzymatic digestion methods. *The American Journal of Clinical Nutrition*, vol. 33, no. 3, pp. 677-686. http://dx.doi.org/10.1093/ajcn/33.3.677. PMid:6986763.

BOUTLER, D., THURMAN, D.A. and TURNER, B.L., 1966. The use of disc electrophoresis of plant proteins in systematics. *Taxon*, vol. 15, no. 4, pp. 135-143. http://dx.doi.org/10.2307/1217532.

BRADFORD, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3. PMid:942051.

CASEY, R.C., DOMONEY, C. and ELLIS, N., 1986. Legume storage proteins and their genes. In: B.J. MIFLIN, ed. *Oxford*

Surveys of Plant Molecular and Cell Biology. Oxford: Oxford University Press, vol. 3, pp. 1.

CHIBBAR, R.N., AMBIGAIPALAN, P. and HOOVER, R., 2010. Molecular diversity in pulse seed starch and complex carbohydrates and its role in human nutrition and health. *Cereal Chemistry*, vol. 87, no. 4, pp. 342-352. http://dx.doi.org/10.1094/CCHEM-87-4-0342.

DAS, S. and MUKARJEE, K.K., 1995. Comparative study on seed proteins of *Ipomoea. Seed Science and Technology*, vol. 23, pp. 501-509.

DHAWAN, K., MALHOTRA, S., DAHIYA, B.S. and SINGH, D., 1991. Seed protein fractions and amino acid composition in gram (Cicer arietinum). *Plant Foods for Human Nutrition*, vol. 41, no. 3, pp. 225-232. http://dx.doi.org/10.1007/BF02196390. PMid:1924186.

FAOSTAT [online], 2011. [viewed 12 December 2011]. Available from: http://faostat.fao.org/site/567/DesktopDefault.aspx

FERREIRA, J.J., ALVAREZ, E., FUEYO, M.A., ROCA, A. and GIRALDEZ, R., 2000. Determination of the out crossing rate of *Phaseolus vulgaris* L. using seed protein markers. *Euphytica*, vol. 113, no. 3, pp. 259-263. http://dx.doi.org/10.1023/A:1003907130234.

FRIMPONG, A., SINHA, A., TARAN, B., WARKENTIN, T.D., GOSSEN, B.D. and CHIBBAR, R.N., 2009. Genotype and growing environment influence chickpea (*Cicer arientinum* L.) seed composition. *Journal of the Science of Food and Agriculture*, vol. 89, no. 12, pp. 2052-2063. http://dx.doi.org/10.1002/jsfa.3690.

GEERVANI, P., 1991. Utilization of chickpea in India and scope for novel and alternative uses. In: *Proceedings of a Consultants Meeting*, 27-30 March 1989. Andhra Pradesh, India: ICRISAT, pp. 47-54.

GEPTS, P. and BLISS, F.A., 1988. Dissemination pathways of common bean (*Phaseolus vulgaris, Fabaceae*) deduced from phaseolin electrophoretic variability. II. Europe and Africa. *Economic Botany*, vol. 42, no. 1, pp. 86-104. http://dx.doi.org/10.1007/BF02859038.

GEPTS, P., KMIECIK, K., PEREIRA, P. and BLISS, F.A., 1988. Dissemination pathways of common been (*Phaseolus vulgaris, Fabaceae*) deduced from phaseolin electrophoretic variability. 1. The Americas. *Economic Botany*, vol. 42, no. 1, pp. 73-85. http://dx.doi.org/10.1007/BF02859036.

GHAFOOR, A., AHMAD, Z., QURESHI, A.S. and BASHIR, M., 2002. Genetic relationship in *Vignamungo*(L.) Hepper and *V. radiata*(L.) R. Wilczek based on morphological traits and SDS-PAGE. *Euphytica*, vol. 123, no. 3, pp. 367-378. http://dx.doi.org/10.1023/A:1015092502466.

GHAFOOR, A., GULBAAZ, F.N., AFZAL, M., ASHRAF, M. and ARSHAD, M., 2003. Inter-relationship between SDS-PAGE markers and agronomic traits in chickpea (*Cicer arietinum* L.). *Pakistan Journal of Botany*, vol. 35, no. 4, pp. 613-624.

HAMEED, A., SHAH, T.M., ATTA, B.M., IQBAL, N., HAQ, M.A. and ALI, H., 2009. Comparative seed storage protein profiling of kabuli chickpea genotypes. *Pakistan Journal of Botany*, vol. 41, no. 2, pp. 703-710.

HUISMAN, J. and POEL, A.F.B., 1994. *Aspects of the nutritional quality and use of cool season food legumes in animal feed*. Dordrecht, The Netherlands: Kluwer Academic Publishers, pp. 53-76 http://dx.doi.org/10.1007/978-94-011-0798-3_2.

HULSE, J.H., 1991. Nature, composition and utilization of grain legumes. In: *Uses of tropical Legumes: Proceedings of a Consultants*, 27-30 March, Patancheru, India. Patancheru, India: ICRISAT, pp. 11-27.

HUSSAIN, A., IQBAL, S.M., GHAFOOR, A. and AYUB, N., 2010. Biochemical variability among the isolates of *sclerotiumrolfsii*of chickpea in pakistan. *Pakistan Journal of Phytopathology*, vol. 22, no. 1, pp. 34-39.

IQBAL, S.H., GHAFOOR, A. and AYUB, N., 2005. Relationship between SDS-PAGE markers and Ascochyta blight in Chickpea. *Pakistan Journal of Botany*, vol. 37, pp. 87-96.

JAMBUNATHAN, R. and SINGH, U., 1981. Studies on desi and kabuli chickpea (*Cicer arietinum* L.) cultivars. 3. Mineral and trace element composition. *Journal of Agricultural and Food Chemistry*, vol. 29, no. 5, pp. 1093-1095. http://dx.doi.org/10.1021/jf00107a050. PMid:7309995.

JAVED, A., GHAFOOR, A. and ANWAR, R., 2004. Seed storage proteins electrophoresis in groundnut for evaluating genetic diversity. *Pakistan Journal of Botany*, vol. 36, pp. 87-96.

JAVID, A., GHAFOOR, A. and ANWAR, R., 2004. Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pakistan Journal of Botany*, vol. 36, pp. 87-96.

JUKANTI, A.K., GAUR, P.M., GOWDA, C.L. and CHIBBAR, R.N., 2012. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.). *British Journal of Nutrition*, vol. 108, no. 1, suppl. 1, pp. 11-26. http://dx.doi.org/10.1017/S0007114512000797. PMid:22916806.

KAGA, A., TOMOOKA, N., EGAWA, Y., HOSAKA, K. and KAMIJIMA, O., 1996. Species relationship in the subgenus *Ceratotropis*(genus *Vigna*) as revealed by RAPD analysis. *Euphytcia*, vol. 88, no. 1, pp. 17-24. http://dx.doi.org/10.1007/BF00029261.

KHAN, M.K., 1990. Production and utility of chickpea (*Cicerarietinum*L.) in Pakistan. *Progressive Farming*, vol. 10, pp. 28-33.

LADIZINSKY, G. and HYMOWITZ, T., 1979. Seed protein electrophoresis in taxonomic and evolutionary studies. *Theoretical and Applied Genetics*, vol. 54, no. 4, pp. 145-151. http://dx.doi.org/10.1007/BF00263044. PMid:24310336.</jrn>He,

LAEMMLI, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, vol. 227, no. 5259, pp. 680-685. http://dx.doi.org/10.1038/227680a0. PMid:5432063.

LIOI, L.F., SPARVOLI, F. and BOLLINI, R., 1999. Variation and genomic polymorphism of lectin-related protein in Lima Bean (*Phaseoluslunatus*L.) seed. *Genetic Resources and Crop Evolution*, vol. 46, no. 2, pp. 157-182. http://dx.doi.org/10.1023/A:1008630330008.

MANSFIELD, D. and BAERLOCHER, M.O., 2005. Free amino acids. In: M.A.S. GRAÇA, F. BÄRLOCHER and M.O. GESSNER, *Methods to study litter decomposition*. Neatherlands: Springer, Chap. 10, pp. 69-74. http://dx.doi.org/10.1007/1-4020-3466-0_10.

MEHRANI, P., 2002. *Genetic diversity in local and exotic pea (Pisaum sativum L.) germplasm for morphological traits and SDS-PAGE markers. M. Phil*. Islamabad, Pakistan: Department of Biological Sciences, Quaid-I-Azam University, 104 p. Thesis.

MILAN–CARILLO, J., REYES-MORENO, C. and ARMIENTA-RODELO, E., 2000. Physicochemical and nutritional characteristic

of fresh and hardened chickpeas (*Cicer arietinum*). *LWT*, vol. 33, pp. 117-123. http://dx.doi.org/10.1006/fstl.1999.0620.

MORENO, M. and CUBERO, J.I., 1978. Variation in *Cicer arietinum L. Euphytica*, vol. 27, no. 2, pp. 465-485. http://dx.doi.org/10.1007/BF00043173.

MURPHY, R.W., SITES, J.W., BUTH, D.G. and HAUFLER, C.H., 1990. Protein isozymeseletrophoresis. In: D.H. HILLIS and C. MORITZ, eds. *Molecular systematic*. Sunderland, MA: Sinnauer Association, pp. 45-126.

MURRAY, D.R., 1979. A storage role for albumins in pea cotyledons. *Plant, Cell & Environment*, vol. 2, no. 3, pp. 221-226. http://dx.doi.org/10.1111/j.1365-3040.1979.tb00073.x.

NAJMA, A., IQBAL, S.M. and GHAFOOR, A., 2005. Relationship between SDS-PAGE markers and ascochyta blight in chickpea. *Pakistan Journal of Botany*, vol. 37, no. 1, pp. 87-96.

NAKAJIMA, K., 1994. Biotechnology for crop improvement in Japan In: *Biotechnology application in agriculture in Asia and Pacific*. Tokyo, Japan: Asian Productivity Organization pp. 87-107.

NEI, N. and LI, W., 1979. Mathematical model for studying genetic variations in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 76, no. 10, pp. 5269-5273. http://dx.doi.org/10.1073/pnas.76.10.5269. PMid:291943.

NISAR, M., GHAFOOR, A., KHAN, M.R., AHMAD, H., QURESHI, A.S. and ALI, H., 2007. Genetic diversity and geographic relationship among local and exotic chickpea germplasm. *Pakistan Journal of Botany*, vol. 39, no. 5, pp. 1575-1581.

PANDE, S.K.H.M.S. and KISHORE, G.K., 2005. Ascochyta blight of chickpea: Biology, pathogenicity, and disease management. *Australian Journal of Agricultural Research*, vol. 56, pp. 317-332. http://dx.doi.org/10.1071/AR04143.

PARKER, P.G., SNOW, A.A., SCHUG, M.D., BOOTON, G.C. and FUERST, P.A., 1998. What molecules can tell us about populations: choosing and using a molecular marker. *Ecology*, vol. 79, no. 2, pp. 361-382. http://dx.doi.org/10.2307/176939.

RAO, R., VAGLIO, D.M., MDU, P. and MONTI, L., 1992. Identification of *Vigna* ssp. Through specific seed storage polypeptides. *Euphytica*, vol. 62, no. 1, pp. 39-43. http://dx.doi.org/10.1007/BF00036085.

SARWAR, G. and PEACE, R.W., 1986. Comparisons between true digestibility of total nitrogen and limiting amino acids in vegetable proteins fed to rats. *The Journal of Nutrition*, vol. 116, no. 7, pp. 1172-1184. http://dx.doi.org/10.1093/jn/116.7.1172. PMid:3746456.

SATIJA, D.R., ADRASH, B., GUPTA, S.K. and BALA, A., 2002. Genetic diversity in relation to protein and protein fractions in chickpea (*Cicer arietinum* L.). *Crop Improvement*, vol. 29, no. 2, pp. 122-135.

SIMON, C.J. and MUEHLBAUER, F.J., 1997. Construction of chickpea linkage map and its comparison with the map of pea and lentil. *Journal of Heredity*, vol. 88, no. 2, pp. 115-119. http://dx.doi.org/10.1093/oxfordjournals.jhered.a023068.

SINGH, U. and JAMBUNATHAN, R.., 1982. Distribution of seed protein fractions and amino acids in different anatomical parts of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* L.). *Plant Foods for Human Nutrition*, vol. 31, no. 4, pp. 347-354. http://dx.doi.org/10.1007/BF01094046.

THAKARE, R.G., GADGIL, J.D. and MITRA, R., 1987. Origin and evolution of seed proteins genes in Vigna mungo and V. Radiata. In: Proceedings of the 2nd International, Mungbean Symposium. Banskok, Thailand. Mungbean: AVRDC. pp. 47-52.

VALIZADE, M., 2001. Seed storage protein profile of grain legumes grown in Iran, using SDS-PAGE. *Journal of Agricultural Science and Technology*, vol. 3, pp. 287-292.

WORLD HEALTH ORGANIZATION – WHO and FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS – FAO, 1973. *Energy and Protein Requirements*. Rome: FAO/WHO. Report of FAO Nutritional Meeting Series, no. 52.

ZIA-UL-HAQ, M., IQBAL, S., AHMAD, S., IMRAN, M., NIAZ, A. and BHANGER, M.I., 2007. Nutritional and compositional study of desi chickpea (Cicer arietinum L.) cultivars grown in Punjab, Pakistan. *Food Chemistry*, vol. 105, no. 4, pp. 1357-1363. http://dx.doi.org/10.1016/j.foodchem.2007.05.004.

Supplementary Material

Supplementary material accompanies this paper.

Supplementary Table 1: Mean concentration values for total seed storage proteins of sixteen different chickpea varieties. Data with different superscripts within the column represents significant difference at $p<0.05$.

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