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GENETIC DIVERSITY FOR SOME NUTRITIVE TRAITS OF CHICKPEA (*Cicer arietinum* L.) FROM DIFFERENT REGIONS IN KOSOVA

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ABSTRACT

Chickpea (Cicer arietinum L.) is an important pulse crop with a wide range of potential nutritional benefits because of its chemical composition. Seeds from seven chickpea genotypes were evaluated for their proximate analysis for quantitative traits, protein, oil and mineral composition. The experimental material comprising 7 genotypes of chickpea was grown in a Randomized Complete Block Design (RCBD) with three replications during the vegetation periods 2013 and 2014 in Ferizaj locality, in the southern part of Kosova 35 km away from capital city Pristina. The results showed that there was wide variation among chickpea genotypes. Grain yield was 22.72 g plant^{-1} while protein was 28.85 g/100 g. Genotypes FBV-RA and FBV-FE exhibited the highest protein content (mean = 29.70 g/100 g and 29.66 g/100 g, respectively). Oil content was 2.878 g/100 g. Also genotypic differences for mineral content were statistically significant. It was observed that the first three principal components explained 96.3% of the variability. Based on cluster analyses, the chickpea genotypes were classified into four main groups. Generally, results and findings suggest to be a great chance of genetic improvements in chickpea.

Keywords: Chickpea, Cicer arietinum, mineral content, oil, protein

INTRODUCTION

The knowledge of genetic diversity is a useful 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. Chickpea (*Cicer arietinum* L.) belongs to the family *Fabaceae*. The genus *Cicer* L. comprises 49 taxa with 40 wild perennials, 8 wild annuals and one annual cultivated species (Toker et al. 2014; Smykal et al. 2015). Chickpea is the second most important food legume in the world in terms of area (13.5 million hectares) and production (13.1 million tons) in 2013 after beans (FAOSTAT 2016). It was domesticated in association with other crops as part of the evolution of agriculture in the Fertile Crescent 12 000–10 000 years ago (Zohary and Hopf 2000). The crop certainly originates from Turkey and Syria (Ladizinsky and Adler 1976; Toker 2009), and its progenitor (*C. reticulatum* Ladiz.) is an endemic species to South and East Turkey (Toker et al. 2014). The major chickpea producing countries in the world are India, Australia, Turkey, Pakistan and Iran. In Europe, the crop was perhaps diffused by the Spanish and Portuguese travelers (van der Maesen 1987). Today the crop is widely distributed, being grown in over 33 countries in the world including South Asia, West Asia, North and East Africa, Southern Europe, North and South America and Australia (Anbessa et al. 2007). Chickpea is mainly produced in arid or semiarid environments. Due to several morphological and physiological advantages, the crop can effectively cope with drought conditions (Neugschwandtner et al., 2013). Chickpea yields, yield components and protein contents are affected by production system and fertilization regime (Caliskan et al. 2013). In part of the Balkan area, the crop was cultivated earlier, also in Kosovo. Here it was used after roasting for preparation of white coffee like "*surogat*". Seeds of chickpea were also used for food in the form of rolls. In the region of Dalmatia, this crop is still cultivated but not in large area (Gagro 1997). There it is known as "*Çiçeron*", which is

probably synonymous of the Illyricum word *'qe çerohet'*. Dalmatia previously has been colonized by the Illyrians. Perhaps this word is also connected with Albanian languages and today has the same sense (Aliu et al. 2010 and 2015). Although chickpea is not a common crop in Central Europe, it could provide an alternative for food and feed protein production in the face of climate change. Recently, the plant has been tested in semiarid regions of Austria (Neugschwandtner et al. 2013), the Northern Great Plains in North America (Miller et al. 2002) and in western Canada (Anbessa et al. 2007). Furthermore, the adoption of chickpea in Central Europe could lead to crop diversification and improved productivity of sustainable agricultural systems as legumes satisfy a bulk of their N demand from atmospheric N through symbiosis with N fixing rhizobia (Neugschwandtner et al. 2014). In agronomic practices it is used in crop rotation (Sahin and Gecit 2006) for its atmospheric nitrogen fixation ability and maintenance of soil fertility (Caliskan et al. 2013). From the nutritive values, one of the oldest groups of agricultural plants are food legumes which are the second most important human's food supply after the cereal grains. Their grain contains 38 to 59% carbohydrate, 4.8 to 5.9% oil, 3% ash, 3% fiber, 0.2% calcium, and 0.3% phosphorus (Hulse, 1991). A number of vital minerals like calcium, magnesium, zinc, potassium, iron, phosphorus and vitamins thiamine and niacine required by human body are also found in chickpea (Zia-Ul-Haq et al. 2007). Chickpea packs two to three times more protein (Tonk et al. 2010) and oil in their seeds compared to cereals (Tonk et al. 2010). This crop has been named as "poor man's meat and rich man's vegetable". Therefore, an experiment was conducted to assess genetic variation, trait association and significant contribution of each trait towards yield. Also nutritionally valuable minerals and their contribution were included to identify divergent parents for future hybridization programmes for yield and quality improvement.

MATERIALS AND METHODS

Site conditions

Kosovo has a central geographic position on the Balkan Peninsula. It lies between 41°50'58" and 43°51'42" northern latitude and 20°01'30" and 21°48'02" eastern longitude. The experiment was carried out in Ferizaj, 21°06'25" Longitude, 42°22'48" Latitude, on altitude: 611 m a.s.l. in southern part of Kosovo during years 2013 and 2014. All genotypes were collected in different parts of Kosova (Table 1) from our expedition during year 2012. This region has a continental climate, rainfall averaging about 616.9 mm per year and mean annual temperature of about 11.9 °C. Summer temperatures exceed 35 °C resulting in high evapotranspiration (HMIK, 2014). The area is characterized by flat topography. The soil is classified as of alluvial origin and rich in calcareous sediments (pH 7.1). The temperature was considerable higher in 2013 than in 2014. Total rainfall in 2013 was 566.5 mm and 743.2 mm in 2014. Average temperature in 2013 and 2014 was 11.9°C and 11.3°C, respectively. Monthly precipitation for years 2013 and 2014 was highly above average in April (40.4 and 52.30 mm) and May (122.3 and 154.2 mm). Table 2 shows the long-term average monthly temperatures and precipitation an observations during years 2013-2014.

Table 1. Name and geographical origins of investigated genotypes

Genotype number	Genotype name	Maturity Type	Type	Longitude	Latitude	Altitude	Origin
1	FBV-XE	Late (L)	Kabuli	20°43'59"	42°16'49"	326	Xerxe
2	FBV-SU	Semi late (SL)	Kabuli	20°46'08"	42°05'06"	356	Suhareka
3	FBV-PZ	Semi late (SL)	Kabuli	20°41'59"	42°14'15"	379	Prizren
4	FBV-SU-2	Early (E)	Kabuli	20°49'48"	42°20'25"	412	Suhareka
5	FBV-FE	Late (L)	Kabuli	21°13'32"	42°20'12"	562	Ferizaj
6	FBV-RA	Semi late (SL)	Kabuli	20°26'39"	42°45'54"	580	Rahovec
7	FBV-MA	Semi early SE)	Kabuli	20°42'26"	42°36'46"	380	Malisheve

Table 2. Long term average monthly temperature and precipitation and observations during 2013-2014 growing seasons.

Month	Precipitation (mm)			Temperature (^{0}C)		
	2013	2014	LTA*	2013	2014	LTA
January	38.70	34.50	36.60	1.60	-0.10	0.75
February	5.80	12.50	9.15	3.90	2.50	3.20
March	70.30	74.50	72.40	6.60	6.20	6.40
April	40.40	52.30	46.35	12.80	11.30	12.05
May	122.30	154.20	138.25	16.70	15.30	16.00
June	55.30	65.50	60.40	19.00	20.00	19.50
July	32.60	68.90	50.75	21.60	21.10	21.35
August	21.20	71.50	46.35	23.40	22.50	22.95
September	56.60	58.50	57.55	16.80	15.30	16.05
October	64.80	48.70	56.75	12.60	12.50	12.55
November	42.60	49.80	46.20	7.40	7.30	7.35
December	15.90	52.30	34.10	0.70	2.10	1.40
Average	566.50	743.20	54.57	11.90	11.30	11.63

*LTA - Long Term Average

Experimental design

The experimental material comprising 7 genotypes of chickpea was grown during growing seasons of 2013 and 2014 in a Randomized Complete Block Design (RCBD) with three replications. The seeds were sown in row distance of 40 cm. The plot sizes were 2 × 1.5 m. Seeds were placed at 2-3 cm depth in each row keeping 15 cm distance between two hills. Two seeds were sown in each hill. The excess plants were thinned out keeping one plant in each hill 15 days after sowing (DAS). The plots were fertilized with 30 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O. The grain yields (g $plant^{-1}$) were measured by harvesting each plot at crop maturity. Six plants were randomly chosen from each plot to measure the agronomic traits including biological shoot yield per plant (g), 100 seed weight (g), shoot biomass without pods (g), grain yield (g), and harvest index (%).

Laboratory studies

At harvest time, six random plants were selected in each plot and grains were carefully removed by hand. From each plant, an equal number of grains was taken from each plot, mixed together in order to form a representative sample and then subjected to proximate analyses in the laboratory. The grains were grounded to form a fine powder. The chemical analyses (g/100 g) included protein content (PC), cellulose content (CC), fat content (FC) and mineral content (MC). Analyses were based on standard methods: PC was determined based on Kjeldahl-N, while FC was determined by extraction using Soxhlet method (using petroleum ether at boiling point 40-60°C). The cellulose was determined by the method of Updegroff (1969). Plant fiber was soaked in acetic acid and nitric acid to remove lignin, hemicelluloses and xylans. Cellulose in the sample was hydrolyzed to sugars and results are expressed as percent on dry weight basis. MC was determined by dry incineration at 550°C for 4-6 hours. The mineral elements Fe, Ca, Zn, Mg, Na, Mn, K and Cu in (mg kg^{-1}) were determined from the ash that was subsequently digested in HCl (ratio 1:4) and analyzed with an atomic absorption spectrophotometer (1100 B Perkin-Elmer, Germany). The carbohydrate content (on dry weight basis) was calculated by the differences:

[100- (PC + FC + MC + crude fiber)].

Statistical analysis

All statistical analyses were performed using SPSS 15.0 (SPSS, Inc., Chicago, USA). The analysis of variance and Duncan's Multiple Range Test (DMRT) were performed to test factorial effects and differences between means. Mean values of the agronomic traits for genotypes were standardized and used for computing Euclidean distances between them. Principal component analyses (PCA) and cluster analyses were used to obtain Euclidean distances between genotypes and to characterize the relation to the most discriminating traits. A P value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Mean squares for different agronomic traits which are included in our investigations are presented in Table 3. All sources of variance were found as statistically significant for biological yield, shoot biomass, grain yield, seed protein, oil, cellulose, carbohydrates content and harvest Index, while interaction between genotypes (G) x year (Y) were not statistically significantly for some traits. The variability among genotypes was high for biological yield (BY), plant biomass without pods (PB), grain yield (GY), 100-seed weight and harvest index (HI). The average values for biological yield were 75.82 g $plant^{-1}$ and shoot biomass without pods (53.18 g $plant^{-1}$). The differences between them were 22.64 g $plant^{-1}$. Talebi et al. (2008). For plant biomass we found on average 20.1 g $plant^{-1}$. The chickpea genotype FBV-XE had significantly higher biological yield (118.5 g $plant^{-1}$), while FBV-SU-2 realized the lowest value for biological yield (57.65 g $plant^{-1}$). Thus the range was 65.85 g $plant^{-1}$. The final grain yield (g $plant^{-1}$) in chickpea genotypes is considered to be a combined effect of various yield components, like number of seeds per plant, number of pods per plant, number of grains per pod, weight of grains per pod and 100-seed weight could be the responsible traits for yield change (Toker 2004; Toker and Cagirgan 2004). The mean value of grain yield across all genotypes was 22.72 g $plant^{-1}$ and that was relatively high and may be could guarantee a high yield. Results of the grain yield exhibited significant differences among genotypes (Table 3). FBV-FE had the highest grain yield (30.35 g $plant^{-1}$), while FBV-SU-2 showed the lowest grain yield (18.57 g $plant^{-1}$). The range was 11.78 g $plant^{-1}$ (51.84 %). Different results of grain yield were reported also from other researchers. Talebi et al. (2013) reported different results for grain yield which ranged from 14.75 to 23.75 with a mean value of 17.9 g $plant^{-1}$. Caliskan et al. (2013) also reported different results for grain yield with an average value of 23.79 g $plant^{-1}$. 100-seed weight (g) results ranged from 18.02 to 24.87 g with a mean value of 21.13 g. The physiological efficiency of chickpea plants to convert the total dry matter to grain yield can be estimated by calculating the harvest index (HI). The value of HI varied from 20.21 % (FBV-XE) to 38.04 % (FBV-TE), with significant differences among genotypes. HI obtained by Caliskan et al. (2013) varied from 21.1 % to 35.1 %. Substantial agronomic genetic diversity is given in Table 4.

Proximate compositions of the different chickpea genotypes are shown in Table 5. Significant differences were observed among the genotypes. As shown in Table 5, carbohydrates represent the main fraction of chickpea seed composition (43.65 – 46.32 g/100 g). In the present study, the mean total carbohydrate value of seven chickpea genotypes was 44.64% ± 0.36%, lower than 67.8% total carbohydrates obtained by Shad et al. (2009). Protein mean value was 28.85 g/100 g. Genotypes FBV-RA and FBV-FE exhibited the highest protein content (mean = 29.70 g/100 g and 29.66 g/100 g, respectively).

Similarly, El-Adawy (2002) reported differences among genotypes in protein content (23.64 g/100 g for Egyptian genotypes and 18.5 g/100 g for Brazilian genotypes). Oil content mean value of the evaluated genotypes was 2.88 ± 0.02 g/100 g. It ranged from 2.75 to 2.95 g/100 g. There were significant differences among the chickpea genotypes for both the protein and oil content. Different results were reported by De Almeida Costa et al. (2006) who at some different chickpea genotypes obtained seed oil content up to 9.01 g/100 g. The cellulose content ranged from 8.51 g/100 g to 8.86 g/100 g in genotypes, showing significant differences between them. FBV-RA had the highest cellulose content (mean = 8.86 g/100 g) and FBV-SU contained the lowest value (mean = 8.51 g/100 g).

Table 3. Mean square of some traits in chickpea genotypes

Sources of variation	Biological yield	Shoot biomass	Grain yield	100 seed weight	Protein content	Oil content	Cellulose content	Carbohydrate content	Harvest index
Genotypes (G)	5.03**	5.024**	4.23**	8.36**	21.15**	7.05**	5.90**	143.20**	13.7**
Year (Y)	$1.81^{N.S}$	9.397**	3.05**	18.41**	22.98**	14.75**	19.84**	266.4**	10.78**
G xY	$0.01^{N.S}$	$0.20^{N.S}$	$0.96^{N.S}$	3.54**	$0.59^{N.S}$	1.46*	4.12**	$0.90^{N.S}$	3.88**
Error	13.21	1.09	2.08	12.54	7.06	1.21	0.007	0.005	0.07
Total	39009.7	41962.8	1303.16	1299.03	50.06	0.378	0.7432	36.28	1404.60

*, ** is significant at p=0.05 and p=0.01 respectively.

Table 4. Agronomic traits of chickpea genotypes

Genotypes	Biological yield (g plant^{-1})	Shoot biomass (g plant^{-1})	Grain yield (g plant^{-1})	100 seed weight (g)	Harvest Index (%)
FBV-XE	118.50^{a}	94.75^{a}	23.75^{ab}	23.94^{ab}	20.21^{g}
FBV-SU	67.15^{b}	45.83^{ab}	21.46^{ab}	18.92^{bc}	31.95^{d}
FBV-PZ	79.28^{b}	58.44^{ab}	20.84^{b}	18.02^{c}	26.28^{f}
FBV-SU-2	57.65^{b}	39.08^{b}	18.57^{b}	18.95^{bc}	32.21^{c}
FBV-FE	80.36^{ab}	50.10^{ab}	30.35^{a}	23.49^{abc}	37.76^{b}
FBV-RA	67.30^{b}	46.23^{ab}	21.07^{ab}	24.87^{a}	31.30^{e}
FBV-MA	60.48^{b}	37.47^{b}	23.01^{ab}	19.71^{abc}	38.04^{a}
Mean	75.82	53.13	22.72	21.13	31.10
CV (%)	27.33	36.96	16.51	13.49	3.09

Values within individual columns indicated by at least one equal letter are not significantly different at 0.05 probability level.

Table 5. Proximate (g/100 g) and mineral (mg/kg) seed composition in chickpea genotypes

Genotypes	Protein content	Oil content	Cellulose content	Carbohydrate content	Micronutrients					Macronutrients		
					Zn	Fe	Cu	Mn	Na	Mg	Ca	K
FBV-XE	28.87^{c}	2.91^{ab}	8.61^{cd}	44.51^{d}	24.06^{f}	21.42^{e}	2.7^{d}	5.68^{f}	21.68^{f}	556.5^{f}	376.89^{c}	5232.43^{d}
FBV-SU	27.46^{c}	2.95^{a}	8.51^{d}	46.32^{a}	27.03^{d}	26.3^{d}	1.91^{f}	9.35^{d}	42.03^{c}	570.23^{e}	403.93^{b}	5472.66^{b}
FBV-PZ	27.87^{c}	2.84^{b}	8.66^{bc}	45.39^{b}	24.86^{e}	26.19^{d}	2.04^{e}	9.55^{d}	37.16^{e}	557.6^{f}	441.5^{a}	5378.37^{c}
FBV-SU-2	29.56^{ab}	2.75^{c}	8.86^{a}	43.94^{e}	42.91^{b}	32.34^{a}	2.69^{d}	15.4^{a}	48.6^{a}	696.26^{a}	336.7^{d}	5877.63^{a}
FBV-FE	29.66^{b}	2.96^{a}	8.81^{ab}	43.65^{g}	43.58^{a}	31.29^{b}	3.52^{b}	13.53^{c}	38.56	654.64^{d}	303.76^{e}	5930.47^{a}
FBV-RA	29.70^{a}	2.83^{b}	8.86^{a}	43.83^{f}	42.26^{c}	31.13^{b}	3.2^{c}	14.6^{b}	43.6^{b}	667.49^{b}	286.83^{f}	5862.33^{a}
FBV-MA	28.8^{b}	2.83^{b}	8.61^{cd}	44.85^{c}	44.09^{a}	30.2^{c}	3.64^{a}	14.57^{b}	42.65^{bc}	657.3^{c}	280.56^{f}	5918.3^{a}
Mean	28.85	2.88	8.7	44.64	35.54	28.41	2.81	11.81	39.18	622.86	347.17	5667.46
CV (%)	3.09	2.65	1.61	2.16	27.07	13.83	24.16	30.91	21.83	9.50	17.86	16.89

*Values within individual columns indicated by at least one equal letter are not significantly different at 0.05 probability level.

Minerals are of great importance in the diet and need to be taken up by food, although they comprise only 4–6% of human bodyweight. Some macro elements are required in amounts greater than 100 mg per day but represent 1% or less of body weight (Insel et al. 2011). More than one third of the world's population is affected by iron (Fe) and zinc (Zn) deficiencies, which are ranked fifth and sixth among the ten most important risk causes of illness and disease in low income countries (WHO 2002). Mg, P, K and Ca were the main mineral elements in chickpeas (Table 5), consistent with information from the USDA (2012) database. In our study, Mg and Ca mean contents were 622.86 mg kg^{-1} and 347.17 mg kg^{-1}, respectively. Ca helps to ease insomnia and helps regulate the passage of nutrients through cell walls, without calcium the muscles in the body cannot contract correctly (Payne 1990). Mn content was higher in FBV-SU-2 (15.4 mg kg^{-1}) and FBV-RA (14.6 mg kg^{-1}) than those of the other genotypes. Fe and Zn are essential micronutrients for humans. The obtained results of Fe and Zn showed higher variation between studied of chickpea genotypes. Overall average value for Fe and Zn was 28.41 mg kg^{-1} and 35.54 mg kg^{-1}, respectively. Neugschwandtner et al. (2015) reported higher concentrations for some elements, i.e. K, Ca, Mg, Cu, Mn, while the observed Zn concentration was similar. Quantities of minerals in cultivated plants are influenced by numerous complex factors including genotype, soil, environmental conditions and nutrition interactions (Simic et al. 2009).

The results of the PCA of the proximate values measured were presented in Table 6. Principal component analysis can determine which of the characters most strongly contribute to the PCA. It was observed that the first component explained 96.3% of the variability among chickpea. The PC2, PC3 and PC4 accounted for additional 2.8%, 0.9% and 0.01 % of the total variation, respectively. The first principal component and second component on maximum values was biological yield (5.94 and 11.86, respectively), while the seed protein was 1.41. The second principal component (PC2) was determined by minerals Zn (0.43), Fe (0.52), Cu (1.81) and Mn (1.92). Results of cluster analysis including similarities are given in Figure 1**.** The seven chickpea genotypes were classified into four main groups. The cluster I comprised only FBV-XE, while cluster II consists of FBV-SU and FBV-RA with minimal differences between them. Cluster III includes the genotypes FBV-SU2 and FBV-TE. Moreover genotypes FBV-FE and FBV-PZ created their own groups alone (cluster IV).

Table 6. Eigenvector values for the first 4 principal components

	Principal components			
Variables	**PC1**	**PC2**	**PC3**	**PC4**
Biological Yield	5.94	11.86	11.71	-0.60
Shoot Biomass	-5.91	-12.19	-11.71	0.56
100 seed weight	-2.09	-0.73	-0.30	1.05
Seed protein content	1.41	0.49	0.14	0.17
Seed oil content	1.01	0.53	-0.03	1.09
Seed cellulose content	1.43	1.20	0.87	-0.05
Seed carbohydrate content	5.10	0.87	0.45	-0.04
Zn	0.57	0.43	-0.17	-0.09
Fe	-2.36	0.52	0.19	1.22
Cu	-1.68	1.81	3.01	0.16
Mn	1.08	1.92	0.30	-0.38
Na	3.32	-0.34	-2.77	0.33
Ca	-4.45	-0.28	2.06	0.08
K	-0.16	0.06	-0.40	0.67
Mg	-0.05	0.37	-0.28	-0.55
Percentage variation	96.3	2.8	0.9	0.01
Percentage cumulative	96.1	99.1	100	100

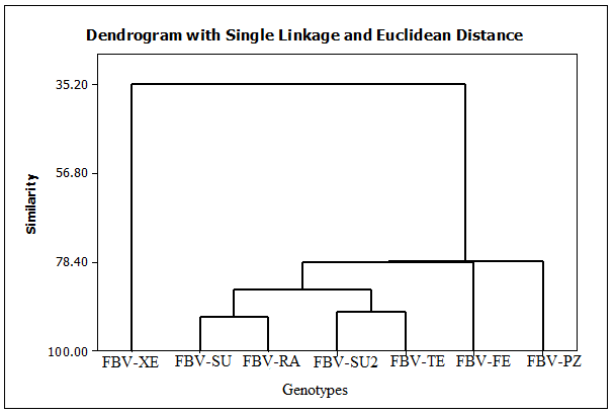

Figure 1. Results of cluster analysis including similarities for chickpea genotypes

CONCLUSIONS

The present study showed a great amount of genetic diversity among the studied genotypes and allows a better knowledge of the chickpea genotypes in different regions of Kosova. It can be concluded that the seed is a good source of protein, oil and different mineral elements. It was observed that the first two components explained more than 99% of the variability among the seven chickpea genotypes evaluated. We can also deduce that these seeds may serve as sources of minerals for human diets. Generally, results and findings from this research suggest a great chance for genetic improvements of chickpea in different breeding programs for the development of desirable genotypes through hybridization.

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