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Assessment of morphological variability and chemical composition of some local pepper (*Capsicum annuum* L.) populations on the area of Kosovo

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ABSTRACT

Seven local pepper populations (*Capsicum annuum* L.) from different geographical regions of Kosovo, were evaluated for morphological traits, chemical composition, and antioxidant contents using standard analytical techniques. All local peppers populations were characterized for different morphological traits from seedling emergence to crop maturity. The total genetic variation for plant height (PH) was 11.72 cm or expressed in relative values was 27.94 %. Average of leaf area (LA) per plant was 2308.38 cm^2, while the lowest value for LA was 1136 cm^2. Yield per plant ranged from 265 to 691 g $plant^{-1}$. The acidity level was ranging from 1.44 to 1.61 %, carbohydrates varied greatly from 4.21 to 6.07 %. Vitamin C (as ascorbic acid) content in fresh fruit ranged from 65.544 to 520.51 mg $100g^{-1}$ of fresh mass. Minerals were of reasonable levels with Fe (15.31 mg kg^{-1}), Ca (216.71 mg kg^{-1}), Na (406.01 mg kg^{-1}), K (1851 mg kg^{-1}), and Zn (5.74 mg kg^{-1}).

Key words: *Capsicum annuum* L.; genetic diversity; antioxidant content; mineral composition

IZVLEČEK

OVREDNOTENJE MORFOLOŠKE SPREMENLJIVOSTI IN KEMIČNE SESTAVE NEKATERIH POPULACIJ PAPRIKE (*Capsicum annuum* L.) NA KOSOVU

S standardnimi analitskimi tehnikami so bile ovrednotene morfološke lastnosti, kemijska sestava in vsebnost antioksidantov sedmih lokalnih populacij paprike (*Capsicum annuum* L.) iz različnih geografskih območij Kosova. Za vse populacije so bile ovrednotene različne morfološke lastnosti od vznika do zrelosti plodov. Celokupna genetska spremenljivost za višino rastlin (PH) je bila 11.72 cm, izražena v relativnih vrednostih je bila 27.94 %. Povprečna listna površina na rastlino (LA) je bila 2308.38 cm^2, najmanjša 1136 cm^2. Pridelek na rastlino je bil med 265 in 691 g na rastlino. Vsebnost kislin je bila med 1.44 in 1.61 %, ogljikovih hidratov med 4.21 in 6.07 %. Vsebnost vitamina C v svežih plodovih je bila med 65.544 in 520.51 mg $100g^{-1}$ sveže mase. Vsebnosti mineralov so bile v običajnih območjih in sicer Fe, 15.31 mg kg^{-1}, Ca, 216.71 mg kg^{-1}, Na, 406.01 mg kg^{-1}, K, 1851 mg kg^{-1}, in Zn, 5.74 mg kg^{-1}.

Ključne besede: *Capsicum annuum* L.; genetska raznolikost; vsebnost antioksidantov; mineralna sestava

1 INTRODUCTION

In the world, several hundred types of peppers are cultivated. Chili pepper (*Capsicum* spp.) is a solaneceous plant, whose centre of origin in Middle America and Mexico is centre of genetic diversity and domestication (Pickersgill, 1971). Csillery (2006) indicates that the first component description of *Capsicum* was given in Hungarian herbal by Dioszegi and Fazekas (1807) cited by Bozokalfa et al., (2009). Pepper (*Capsicum annuum* L.) is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent source of natural colours and antioxidant compounds (Conforti et al., 2007; Deepa et al., 2007). Peppers are one of the main vegetables planted in Kosovo regarding production area and economic importance. The dominate type of pepper production is open field cultivation. Also, the local pepper genotypes in Kosova are rich in diversity with different populations where it has been cultivated for centuries and which is very useable for human food (Aliu et al., 2012; Kaciu et al., 2010). Almost indispensable food, salads, condiment of every Kosovar cuisine is pepper. In Kosovo from total area planted with vegetables, 4449 ha or expressed in

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percentage is 30.68 % are planted with pepper (MAFRD, 2014). Most pepper cultivars currently grown in Kosovo are open pollinated. Some local populations are still grown on many small farms due to consumer demand. For decades, these have been cultivated in different environments and growing techniques. Almost all the cultivars grown are of landraces types which are characterized by a wide range of observable variability. Since their introduction into the world, peppers are cultivated in various environments and a number of different populations were developed (Govindarajan, 1986). In general, they are genetically diverse and well adapted to the locations where they have been developed (Votava et al., 2005). Estimating genetic diversity and determining the relationships between germplasm collections helps ensure germplasm is efficiently collected and managed (Bozokalfa et al., 2009). Data on the level of genetic diversity of a germplasm collection may also increase the efficiency of efforts to improve this crop (Geleta et al., 2005). Pepper fruits are also source of vitamins A, complex B1 and B2, C and minerals such as dietary calcium, iron, magnesium etc. (Bosland,1992). A number of studies report that hot pepper seeds are rich in minerals content (Zou et al, 2015; Jarret et al., 2013; Park et al., 2006). The content of vitamin C in the pepper fruit is higher than in *Citrus* (Finger et al., 2010). The pepper fruit is a rich source of vitamin A, E, C and P in green chilli (Hosmani, 1993; Howard et al., 2000; Marin et al., 2004). They have a high level of vitamins C and E as well as the total of antioxidants is completed by phenolic compounds, which occur in peppers in connection with sugars (Materska et al., 2003; Shotorbani and Jamei, 2013). Antioxidants are beneficial because of their protective roles against multiple diseases such as cancer, anemia, diabetes and cardiovascular diseases. The compounds perform their function by counteracting the oxidizing effects on lipids by scavenging highly reactive oxygen free radicals, the major oxidizing factors for the oxidative modification of low density lipoprotein and nucleic acids (Perucka and Małgorzata, 2007). However, there is little information available about nutritional constituents of hot pepper seeds grown in Kosova. Therefore, the specific objective of this study was to evaluate morphological traits, and antioxidant content in local pepper populations.

2 MATERIALS AND METHODS

2.1 Plant material and collection of samples

Local pepper populations were collected from different bio-climatic regions throughout Kosovo. Kosovo has a central geographic position on the Balkan Peninsula. It lies between latitude 41°50'58 "and 43°51'42" north and 20°01'30" and longitude 21°48'02" east. Seven local peppers populations (LPP) were collected from various agro-climatic regions. The localities of the sampling sites for the Kosova region were: Krusha, Shtime, Lipjan, Viti, Mitrovica, Istog and Dečani. The altitudes of the sampling sites ranged from 306 to 649 m above sea level. The overall climate of Kosovo is a modified continental type, with some elements of a sub-mediterranean climate in the extreme south. Summers are hot with extreme temperatures of up to 37°C. The average annual rainfall is 720 mm (HMIK, 2008). These were selected to represent various geographical areas. (Figure 1).

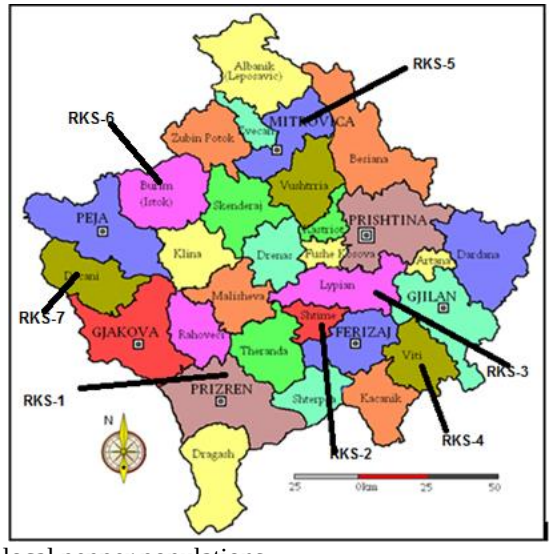

Figure 1: Geographical areas of local pepper populations

A total of seven genotypes within 7 geographical regions were studied under different field conditions over summer 2014. Plant material used in this research was coded as; RKS-A, RKS-B, RKS-C, RKS-D, RKS-E, RKS-F and RKS-G.

Table 1: Pepper populations (*Capsicum* spp.*)* analyzed within their geographical origin

Code	Longitude	Latitude	Elevation	Geographical origin	Local name
RKS-A	020º39´19"	42º18'29"	310	Krusha	Babure
RKS-B	021º32'23"	42º27'27"	577	Shtime	Somborka
RKS-C	021º10'43"	42º29'04"	564	Lipjan	Somborka
RKS-D	021º24'02"	42º21'47"	494	Viti	Dolma
RKS-E	021º54'10"	42º51'47"	521	Mitrovica	Somborka
RKS-F	21°04'05"	42°36'39"	479	Istog	Somborka
RKS-G	20°17'48"	42°31'42"	649	Decan	Somborka

All accessions were characterized for different agro-morphological traits from seedling emergence to crop maturity. All characteristics were measured in the field and laboratory at the normal harvest time. The methodology used to record qualitative values from seedling to harvest was obtained from the descriptor for *Capsicum* from International Plant Genetic Resources Institute (IPGRI 1995). The experimental design was a split plot with randomized complete block with three replications. Plants from local pepper populations were collected in farmer's fields during the period when full maturity of the plants were reached. Local paper populations were collected from different bio-climatic regions throughout Kosovo. To investigate the different traits which were including in our study we collected per location 30 plants x 7 locality = 210 plants (for each location = 3 repetitions x 10 plants/repetition = 30 plants/populations).The following characteristics were measured in Plant breeding laboratory, Faculty of Agriculture, Department of Crop science: plant height (PH), fruit diameter (FD), plant mass without fruits and root (PM), root mass (RM), number of flowers (FN), leaf number (LN), number of fruits (NF), fruit mass (FM), and yield per plant (YP). Leaf area (LA) per plant was measured by planimeter.

*Ascorbic acid extraction***:** The vitamin 'C' as ascorbic acid content (AAC) in fresh pepper fruits was determined by the titratability of 5 g of the blended pulp homogenized with 50 ml of oxalic acid at a concentration of 12 %. The titratable solution consisted of 2.6 sodium indophenol dichlorophenol. Results were expressed in mg of ascorbic acid per 100 g of pulp.

pH: was measured by using a digital pH meter with the application of the electrode directly in to the blended pulp.

The carbohydrates were determined by equipment of refractometer type R 200 (Reichert technologies, USA) precision placing a small sample of blended pulp on the reading prism. Results were expressed as percentage (%).

Water content: 5 g of fruit were taken from each replication, cut into pieces dried in a temperature 105^{o}C in equipment type AD-MF50 until constant mass (90 minutes). Results were expressed in percentage.

Mineral composition: Mineral elements including Fe, Ca, Zn, Mg, Na, K and Cu in (mg kg^{1}) were determined through burning and mineralizing of samples of fruit at 550 ^{o}C for 4 to 6 hours. Samples were digested in HCl and subsequently element concentrations were estimated using an atomic absorption spectrophotometer (1100 B Perkin-Elmer, Germany).

Statistical analyses: all statistical analyses were performed with the SPSS software (version 15.0, SPSS Inc., 2006) to investigate the difference between the populations. Effects of the studied traits were evaluated by ANOVA. Mean separation within columns was done by Duncan's Multiple Range test. In order to assess the differentiation of local pepper populations (LPP's) based on all variables that were measured, the Canonical Discriminant Analyses (CDA) were applied. CDA is a technique for classifying set of observations into predefined classes.

3 RESULTS AND DISCUSSION

The collection of local pepper populations investigated from Kosovo showed considerable variation in morphological and biometric fruit parameters. Plant height (PH) varied from 32.16 to 46.89 cm. The differences between them are 14.73 cm or expressed in percentage is 35.12 %. Results are presented in Table2. According to the analysis of variance (ANOVA) the differences for fruit diameter (FD) between populations were significant. The genetic variation for FD among them was with maximum of 4.73 cm and a minimum of 3.41 cm or differences among populations was 1.32 cm or 31.42 %. Similar range of FD in different pepper genotypes (2.74 to 4.57 cm) was reported by Bozokalfa et al. (2009) and Bassiony et al. (2010) (5.75 till 6.97 cm). The genetic variability for root mass (RM) ranges from 149.30 g plant^{-1} to 100.31 g plant^{-1} which was significant at level of probability of LSD (p = 0.01). Differences for these two populations were 48.99 g plant^{-1} or expressed in relative value was 47.79 %. The significantly higher value for number of flowers (NF) was characterized for population RKS-A with 73.55 flowers per plant, while lower number of flowers was recorded at RKS-C and RKS-E (38.01 flowers per plant). The differences between them were 35.54 flowers per plant or 70.45 %. The leaf number (LN) per plant also showed significant differences among populations. This trait segregated in a manner similar to plant yield. The variability varied from 217.01 to 125.88 leaves per plant, and had significant difference of 91.13 per plant or 52.45 %. Obtained results were in accordance with those obtained by Bassiony et al. (2010), obtaining LN from 158.5 to 191.67 leaves per plant. The leaf area is a one of the crucial factors in photosynthesis. The population RKS-B produced a higher (2308 cm^2 plant^{-1}) leaf area per plant (LA), while lower value was determined for population RKS-E, namely 1136 cm^2 plant^{-1}. The differences between two populations were 1171.56 cm^2 plant^{-1} or expressed in percentage 64.92 %. The results concerning LA are given in Table 2. Also other characters varied substantially. The number of fruits (NF) across pepper populations ranged from 9.44 to 19.66. In our case this trait had an effect an on yield per plant. Plant high yields depend on many factors, the most important factors are the structure of genotypes and environments. Production capacity is a complex character, a result of few morphological components, number of flowers, fruit size, and fruit mass (Madosa et al., 2008). The highest significant contribution (691.44 g) in production (yield) per plant was determined at population RKS-D, followed by RKS -A, a value of 540.66 g plant^{-1}. The major difference among populations is 425.96 g plant^{-1} or 91.34 % respectively. Results are presented in Table 2.

Table 2: Morphological and agronomic traits recorded in different pepper populations

Populations	PH (cm)	FD (mm)	RM g plant^{-1}	PM g plant^{-1}	FN plant^{-1}	LN plant^{-1}	LA cm^2 plant^1	NF plant-1	FM g plant^{-1}	YP g plant^{-1}
RKS-A	46.89±0.16^a	4.42±0.005^a	138.21±0.005^a	116.23±0.015^a	73.55±0.015^a	162.33±0.025^{bc}	1392±0.473^b	15.11±0.055^{ab}	35.71±0.015^{ab}	540.66±0.050^b
RKS-B	42.44±0.10^a	3.96±0.010^b	113.65±0.020^b	86.71±0.010^b	57.44±1.73^b	217.01±0.026^a	2308.±0.451^a	10.66±0.020^{cd}	38.01±0.040^a	402.21±0.412^c
RKS-C	46.55±0.02^a	4.47±0.010^a	135.36±0.010^b	111.76±0.020^a	38.01±0.040^c	178.33±0.015^{ab}	2175±0.666^a	13.33±0.015^{bc}	32.83±0.055^{ab}	431.91±0.196^{bc}
RKS-D	41.66±0.05^a	4.73±0.18^a	149.30±0.005^a	120.14±0.011^a	45.220.043^{bc}	185.11±0.066^{ab}	2009±0.100^a	19.66±0.020^a	35.09±0.035^{ab}	691.44±0.294^a
RKS-E	32.16±0.02^b	3.41±0.005^b	100.31±0.015^b	77.73±0.010^b	38.01±0.040^c	125.88±0.043^c	1136±0.252^b	9.44±0.015^d	27.68±0.11^b	265.48±0.247^d
RKS-F	33.26±0.02^b	3.89±0.010^b	101.21±0.010^b	79.85±0.017^b	37.21±0.015^c	120.25±0.164^c	1212±0.100^b	9.78±0.035^d	25.21±0.09^b	425.96±0.230^{bc}
RKS-G	40.25±0.025^a	4.41±0.010^a	115.25±0.011^b	78.56±0.020^b	44.52±0.010^{bc}	124.78±0.066^c	1354±0.950^{bc}	13.24±0.025^{bc}	31.25±0.020^{ab}	425.96±0.225^{bc}
Mean	40.45±0.025	4.2±0.005	121.89±0.010	95.854±0.017	47.708±0.016	159.09±0.035	1655.54±0.0.493	13.031±0.037	32.25±0.010	454.8±0.133
LSDp=0.05	8.58	1.03	20.86	12.23	10.86	85.05	1239.04	3.37	9.32	132.79
0.01	12.49	1.50	30.36	17.79	15.81	90.34	1802.69	4.90	13.56	193.20

* Values are given as means of three replicates ± SD. Means with different superscript letters within a column are significantly different ($P < 0.05$).

Notes: PH - plant height; FD - fruit diameter; RM-root mass; PMW - plant mass ; FN - flower number; LN - leaf number; LA - leaf area; NF- number of fruits; FMW- fruit mass; YP - yield per plant

The acidity level is from 1.44 to 1.61 % at populations RKS-E and RKS-B, respectively (Table3). Apart from this, these acids make up the energetic reserves and the metabolic reactions that involve the synthesis of pigments, enzymes and other materials and degradation of pectins and celluloses, which are essential in different processes (Antoniali et al., 2007).

Table 3: Average values for some fruit parameters of local pepper populations

Population	Acidity mg 100 g^{-1}	pH values	Carbohydrates %	*AAC content mg 100 g^{-1}	Water content %
RKS-A	1.52±0.005^{b}	5.03±0.037^{b}	4.21±0.010^{c}	65.12±0.011^{a}	91.33±0.147^{b}
RKS-B	1.61±0.006^{a}	5.12±0.010^{b}	6.07±0.068^{a}	58.54±0.045^{d}	90.56±0.168^{b}
RKS-C	1.52±0.010^{b}	5.27±0.020^{a}	4.21±0.015^{c}	62.21±0.121^{b}	93.26±0.0921^{a}
RKS-D	1.53±0.010^{b}	5.24±0.020^{a}	6.14±0.015^{a}	52.51±0.010^{e}	89.71±0.134^{b}
RKS-E	1.44±0.005^{bc}	5.07±0.041^{b}	5.59±0.016^{b}	60.91±0.071^{c}	90.76±0.157^{b}
RKS-F	1.54±0.005^{b}	5.02±0.046^{b}	5.51±0.032^{b}	59.54±0.623^{d}	87.85±0.735^{b}
RKS-G	1.51±0.004^{b}	5.11±0.456^{b}	4.38±0.274^{c}	57.65±0.231^{d}	92.35±0.257^{b}
Average	1.52±0.057	5.15±0.015	5.24±0.041	59.86±0.219	91.12±0.166
LSD *p* = 0.05	0.13	0.11	0.39	0.17	1.33

* AAC- ascorbic acid content. Values are given as means of three replicates ± SD. Means with different superscript letters within a column are significantly different (P < 0.05).

The content of carbohydrates varied greatly within and among LPP's, with values ranging from 4.21 to 6.07 %. The difference among populations for dry fruit mass is 1.86 % or expressed in percentage values was 35.49% higher. AAC is the least complex vitamin found in plants and is synthesized from glucose or other carbohydrates (Kays, 1991). Vitamin C (as ascorbic acid) content in fresh fruit ranged from 65.544 to 520.51 mg 100 g^{-1} of fresh mass. The difference among pepper populations for AAC was 12.61 mg 100 g^{-1} or genetic variation was 21.06 % (Table 3). It was found, that hot pepper cultivars were richer in vitamin C, than the sweet ones. As other studies have shown, the highest or the lowest values of vitamin C in *C. annuum* are dependent on the variety and the maturity stage of the fruits (Khadi et al., 1987; Howard et al., 2000). For example, in the *C. annuum* pepper grown in Turkey, a variation that ranged from 15.2 to 64.9 mg 100 g^{-1} fresh fruit was reported (Topuz and Ozdemir, 2007). And another study conducted in India with the same species showed a variation that ranged from 48.23 to 192.63 mg 100 g^{-1} as reported by Deepa et al. (2006). One of the factors affecting the production of plant biomass is the concentration of mineral elements. The differences among local pepper populations (LPP) are presented in Table 4.

According to the results the highest average values of zinc (Zn) (7.86 mg kg^{-1}) and copper (Cu) (13.9 mg kg^{-1}) was determined LPP coded for population RKS-A. While with the greatest accumulation of iron (Fe) was recorded for RKS-C population with the average value of 23.16 mg kg^{-1}. In our study the other elements including; Ca, Na, K and Mg were on the higher significance results. Results are given in |Table 4.

Table 4: The average mineral content (mg kg^{-1}) in pepper fruits

Populations	Zn^1	Cu^1	Fe^1	Ca^1	Na^1	K^1	Mg^1
RKS-A	7.86±0.043^{a}	13.9±0.17^{a}	14.33±0.13^{b}	236.76±0.59^{ab}	225.76±0.10^{d}	5337.06±4.1^{a}	280.3±0.63^{b}
RKS-B	7.06±0.072^{ab}	0.82±0.21^{e}	14.66±0.13^{b}	216.13±0.40^{b}	238.86±0.24^{c}	2360.83±2.81^{ab}	271.9±0.35^{bc}
RKS-C	4.93±0.077^{bc}	3.26±0.025^{b}	23.16±0.05^{a}	230.2±0.95^{ab}	982.13±0.14^{a}	572.16±1.15^{e}	323.6±0.28^{a}
RKS-D	2.93±0.066^{c}	1.04±0.141^{d}	13.33±0.10^{c}	319.01±0.69^{a}	670.66±0.55^{b}	898.16±0.35^{d}	322.53±0.085^{a}
RKS-E	5.63±0.0921^{b}	1.31±0.005^{c}	13.01±0.095^{c}	83.46±0.13^{c}	247.2±0.17^{c}	1998.76±0.27^{c}	176.01 ±0.41^{c}
RKS-F	4.75±0.0945^{bc}	3.11±0.015^{b}	13.78±0.141^{c}	210.11±0.011^{b}	228.78±0.66^{cd}	895.63±0.78^{d}	275.56±0.092^{bc}
RKS-G	7.02±0.0461^{ab}	0.98±0.016^{e}	14.89±0.21^{b}	221.3±0.10^{b}	248.7±0.53^{c}	901.23±0.51^{cd}	285.23±0.15^{b}
Mean	5.74±0.034	3.490.055±	15.31±0.061	216.71±0.11	406.01±0.45	1851.98±1.24	276.45±1.03

* Values are given as means of three replicates ± SD. Means with different superscript letters within a column are significantly different ($P < 0.05$).

[1]Elemental nutrient composition of local pepper populations (mg kg^{-1})

The canonical discriminant analysis (CDA) of the traits is presented in Figure 2. The first canonical functions described 93.4 % and a second canonical function is 5.6 % of the existing variance. The CDA analysis reported here differentiates LPP's on the basis of similarity. The first group is consisted by RKS-A and RKS-B, second group is consisted by RKS-C and RKS-G. The other populations are separated as individuals, RKS-D, RKS-E and RKS-F. The main elements that can affect the classification to the different groups were the genotype and environment which had influenced on the content of mineral composition and quality traits. The genotype and environmental interaction effects tend to be large when there is a wide variation among genotypes for different traits (Kanf, 1998).

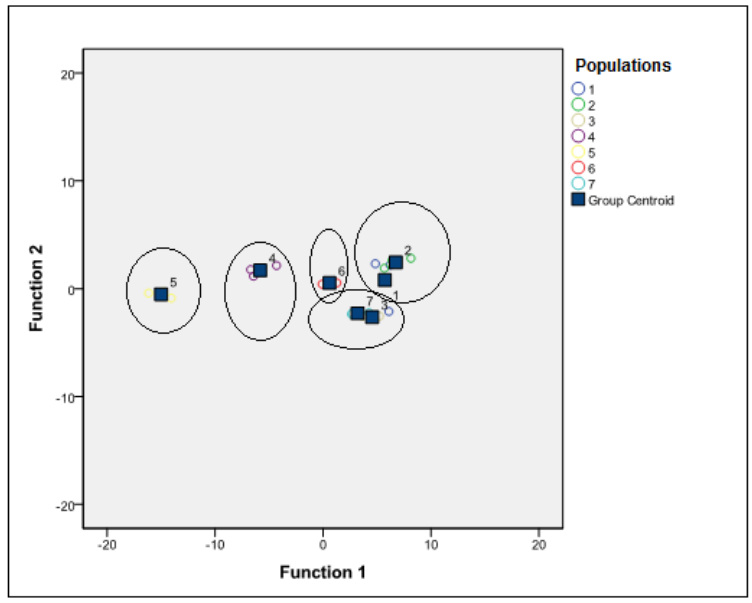

Figure 2: Canonical discriminant function at local pepper populations

4 CONCLUSIONS

The study showed that there was a significant morphological variability among local pepper populations. Some populations are interesting for quantitative traits including leaf area and yield per plant. A high variability was also determined for vitamin C, carbohydrates and mineral composition. The cluster analysis shows that there is an obvious diversity among the populations collected in different geographical regions. The observed morphological diversity among pepper populations is helpful for breeding programs aimed in selecting superior genotypes. These local pepper populations should be included in broader genetic analyses and should be considered as a source of new genetic variability used for the development of inbred lines in the Kosovo breeding program. The evaluation of plant genetic resources has been considered of prime importance, especially in those species having economic importance.

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