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# GENETIC DIVERSITY IN A COLLECTION OF ORNAMENTAL SQUASH (*Cucurbita pepo* L.)

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The aim of this study is collecting and preserving of unique *Cucurbita pepo* germplasm in Serbia. This study also describes the intraspecific variation of *C. pepo* within twenty germplasm accessions. Conservation work aimed to develop a representative core collection of *C. pepo* germplasm, to guide future studies and breeding of its ornamental use. A wide range of *C. pepo* germplasm was collected from diferent parts of the world. Nineteen variables were recorded in 20 varieties to determine the overall degree of polymorphism and to detect similarities among them. Qualitative traits of fruit such is shape, color and texture, showed immense variation. Coefficient of variation were highest for fruit length, fruit weight and number of fruits per plant (CV=56.69-161.32%), while they were the lowest for leaf length (CV=20.65%). Morphological characterization is need to facilitate the use of *C. pepo* varieties in breeding work.

Based on the PCA results, 20 accessions of squash are separate in five groups. Those groups are unique in qualitative and quantitative traits. Knowledge of genetic divergence among varieties is essential for breeding.

*Key words*: *Cucurbita pepo*, genetic resources, PCA, variability

## INTRODUCTION

A Squashes (*Cucurbita* spp.) were among the most widespread, morphologically diverse, and economically important plants grown in the prehistoric gardens and fields of the New World

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(SIMON, 2011). Biogeographical, morphological, molecular, and archaeological data indicate that, by the time of European contact, five different species of squash had been independently domesticated from indigenous wild taxa in regions ranging from south central South America to eastern North America (DECKER-WALTERS *et al.*, 2002). *Cucurbita pepo L.* was the first squash introduced in Europe about 2000 years before new era. Although evidence of this specie is found on many archaeological sites in North America, the earliest material yet recovered comes from the Ocampo Caves (Tamaulipas, Mexico). Primary centre of squash origin is America, with Asia recognized as the secondary centre. Although squash is primarily grown for its fruit consumption, its seeds are a popular snack food, high quality oil source, as well as reproductive material (BERÉNYI and TULOK, 2005; PARIS, 2001). GOHARI *et al.* (2011) study chemical composition of var. styriaka and conclude that pumkin seed was rich in oil and proteins. *C. pepo* L. is one of the most variable species in the plant kingdom.

Fruit types of *C. pepo* are more genetically diverse than are those of any other species in the Cucurbitaceae family, with bicoloured gourds among the most interesting of all gourds. Morphologically, *C. pepo* displays a great diversity of types. The edible forms of this species can thus be grouped into eight morphotypes: Pumpkin, with round fruits; Vegetable Morrow, with short tapered and cylindrical-shaped fruits; Cocozelle, with long bulbous fruits; Zucchini, with uniformly cylindrical-shaped fruits; Acorn, with furrowed, turbinate fruits; Scallop, with flat, scalloped fruits; Crookneck, with narrow, usually curved and warty, necked fruits; and Straightneck, with short-necked or constricted fruits, usually warty. Pumpkin, Vegetable Marrow, Cocozelle and Zucchini, as well as spherical and warty ornamental gourds, correspond to ssp. *pepo*. Scallop, Acorn, Crookneck and Straightneck. Similarly, oviform and pyriform ornamental gourds correspond to ssp. *ovifera* (PARIS, 2001). According to MLADENOVIĆ *et al.* (2011) genotypes of ornamental pumpkins and squashes belong to alternative vegetable crops.

The great variability of types represented in the works of the 16$^{th}$ and 17$^{th}$ century, and the observations of the first European explorers in America, seem to indicate that Pumpkins, Scallops and Acorns were developed under the guidance of Native Americans in pre-Columbian times (PARIS, 2001). The subsequent arrival of *C. pepo* fruits and seeds in Europe lead to an extraordinary variability of new phenotypes through hybridisation and recombination. New cultivars were developed, particularly the elongated forms of ssp. *Pepo*—Vegetable Morrow, Cocozelle and Zucchini. The origin of the Crookneck squash seems to be North American, since it was cultivated in the interior of this continent at the beginning of the 19$^{th}$ century. The commercial cultivars of Straightneck were later derived from Crookneck by outcrossing, selection and breeding (FERRIOL, 2003).

In addition to its nutritional value, cucurbit is used for many other purposes, primarily decorative and artistic. They are excellent table decoration in autumn, and are carved and illuminated by candles during Halloween in the US and other Western cultures.

According to BUCHER (2010), world production of squash in 2010 was 200 000 t, with Europe contributing by 40%, primarily grown in Spain, Romania, France, Greece and Bulgaria, which are the most prominent large-scale pumpkin producers in this region.

The aim of the present work is to analyse the variability of collection and some commercial cultivars belonging to both *C. pepo* subspecies.

## MATERIALS AND METHODS

Twenty *C. pepo* accessions collected from seed houses and sourced from private farmers were included in our investigation. For progeny tests, squash seeds were germinated at 25°C, between two wet sheets of paper. Outdoor planting was performed during 2010, 2011 and 2012 year at the field of Institute of Field and Vegetable Crops in Novi Sad (Serbia). Planting distance between individual plants was 160 × 60 cm.

All accessions were examined for a set of ten quantitative and nine qualitative characters recommended by the European Cooperative Programme for Plant Genetic Resources (ECPGR 2008) descriptors, with minor adaptations, according to importance in horticulture practice. All observations and analyses were carried out on five plants from each accession. All characters were measured in the field at the typical harvest time.

Ten quantitative and nine qualitative characterisics were mesured at twenty genotypes and we gave them next abbreviation: Leaf blade length (LL) in cm, Leaf blade width (LW) in cm, Plant length (L) in cm, Fruit peduncle length (FPL) in cm, Fruit peduncle width (FPW) in cm, Fruit length (FL) in cm, Fruit circumference (FC) in cm, Flesh thickness (FT) in mm, Number of fruits per plant (NF) and Fruit weight (FW) in kg, Plant growth habit (PGH), Peduncle transectional shape (PTS, Fruit shape (FS), Fruit ribs (FR), Predominant fruit skin colour at maturity (PFSC), Secondary fruit skin colour (SFSC), Secondary fruit skin colour pattern (SFSCP), Fruit skin texture (FST) and Flesh colour (FLC).

*Data analysis*. Nine qualitative characters of 20 accessions were quantified according to their graduations. An ANOVA (analysis of variance) was conducted in order to identify potentially significant differences between quantitative characters of accessions. A PCA analysis was used to estimate correlations between characters, generate Eigen values, the percentage of the variation accumulated by PCA and the corresponding load coefficient values (Eigen values are proportional to the amount of total variation among the accessions associated with the axis). The principal components (PC) with Eigen values > 1.0 were selected and the characters for which load coefficient values > 0.6 were considered highly relevant for that PC.

## RESULTS AND DISCUSSION

A large variability in quantitative traits within three-year period was found among 20 accessions of *C. pepo* varieties (Table 1). For example, the mean value of fruit mass for 20 accessions ranged from 0.09 kg to 3.50 kg, fruit length from 5 cm to 32.30 cm and the fruit circumference from 13 cm to 71 cm. Moreover, morphological traits with higher coefficient of variation were usually related to the fruit. For example, the variation coefficient of fruit length was 56.69%, and that of fruit mass was 161.32%.

The investigated data indicated a large range of variability amongst corresponding qualitative characters of 20 *C. pepo* accessions (Table 2). Predominant fruit skin colour at maturity (PFSC) ranged from green (e.g., CP18) to orange (e.g., CP6), secondary fruit skin colour pattern (SFSCP) from speckled (e.g., CP4) to striped (e.g., CP1), fruit skin texture (FST) from smooth (e.g., CP2) to warty (e.g., CP15), and flesh colour (FLC) from white (e.g., CP7) to orange (e.g., CP13).

*Table 1. Quantitative characteristics among 20 accessions of C. pepo (The results were presented as three years averages)*

| Accessions | LL | LW | L | FPL | FPW | FL | FC | FT | NF | FW |
|---|---|---|---|---|---|---|---|---|---|---|
| CP1 | 15,14 | 15,1 | 409 | 4,08 | 1,12 | 7,36 | 14,5 | 5,2 | 31 | 0,22 |
| CP2 | 28,19 | 32,14 | 344 | 5,04 | 1,52 | 27,3 | 30,88 | 17,4 | 18,6 | 0,22 |
| CP3 | 19,16 | 20,06 | 154 | 3,11 | 1,24 | 9,36 | 50,72 | 31 | 7 | 0,3 |
| CP4 | 19,06 | 20,28 | 444 | 4,18 | 1,04 | 6,98 | 27,96 | 21 | 10,8 | 0,16 |
| CP5 | 22,08 | 26,08 | 470 | 15,88 | 2,16 | 20,7 | 61,28 | 29,2 | 4,2 | 0,96 |
| CP6 | 19,34 | 20,18 | 414 | 13,04 | 2,02 | 10,5 | 36,36 | 21,2 | 10,6 | 0,23 |
| CP7 | 14,1 | 16,16 | 406 | 7,98 | 1,04 | 9,24 | 26,7 | 4,4 | 50 | 0,19 |
| CP8 | 19,14 | 29 | 145 | 7,22 | 2,06 | 11,1 | 38,3 | 25,8 | 6,4 | 0,44 |
| CP9 | 24,16 | 22,04 | 446 | 7,96 | 2,12 | 25,38 | 67,76 | 27 | 2 | 3,22 |
| CP10 | 25,14 | 30,06 | 402 | 17,02 | 4,48 | 30,82 | 70,56 | 40,2 | 1,8 | 3,3 |
| CP11 | 21,18 | 23,04 | 348 | 5,06 | 1,12 | 27,64 | 19,72 | 13,2 | 16,2 | 0,18 |
| CP12 | 17,1 | 20,1 | 256 | 6,1 | 1,42 | 12,76 | 39,56 | 22,8 | 5,4 | 0,36 |
| CP13 | 18,1 | 20,06 | 322 | 10,47 | 1,28 | 8,72 | 39,7 | 24,4 | 7 | 0,24 |
| CP14 | 26,62 | 31,06 | 358 | 4,26 | 1,52 | 31,66 | 27,72 | 18,6 | 13,6 | 0,18 |
| CP15 | 22 | 24,02 | 348 | 3,04 | 1,24 | 24,28 | 25,94 | 15,8 | 13,2 | 0,19 |
| CP16 | 14,12 | 16,08 | 408 | 4,14 | 0,66 | 8,7 | 15,3 | 7,5 | 27 | 0,13 |
| CP17 | 14,54 | 23,1 | 440 | 8,96 | 2,88 | 7,9 | 24,04 | 21,6 | 10,6 | 0,2 |
| CP18 | 15,08 | 24,1 | 432 | 9,06 | 4,14 | 8,5 | 20,72 | 22,8 | 7 | 0,21 |
| CP19 | 19,08 | 20,04 | 308 | 3,82 | 1,02 | 5,88 | 28,46 | 19,8 | 4,4 | 0,12 |
| CP20 | 19,12 | 20,14 | 126 | 6,52 | 1,82 | 18,78 | 37,64 | 23,6 | 3,8 | 0,38 |
| Mean | 19,62 | 22,65 | 349 | 7,36 | 1,79 | 15,68 | 35,19 | 20,40 | 12,53 | 0,57 |
| Std | 4,05 | 4,83 | 102,51 | 4,02 | 1,00 | 8,89 | 15,96 | 9,20 | 11,66 | 0,92 |
| C.V. (%) | 20,65 | 21,35 | 29,37 | 54,60 | 55,95 | 56,69 | 45,36 | 45,09 | 93,08 | 161,32 |

Correlation analysis identified both positive and negative correlations between the ten quantitative characteristics, with some significant variations amongst correlations. For instance, leaf length was positively correlated with leaf width, fruit length, fruit circumference, flesh thickness, number of fruits per plant, and fruit weight.

However number of fruits per plant and all other traits, except plant length, were negatively correlated. Positive correlation was found between leaf length and fruit length (Table 3). The aforementioned correlations between characteristics helped determine the most important characters for an identification and comprehensive description of accession.

*Table 2. Qualitative characteristics among 20 accessions of C. pepo*

| Descriptor | Score code | Decriptor state | Frequency (%) |
|---|---|---|---|
| Plant growth habit | 3 | Bushy | 25.0 |
| | 5 | Intermediate | 15.0 |
| | 7 | Prostrate | 60.0 |
| Peduncle transectional shape | 2 | Smoothly angled | 80.0 |
| | 3 | Sharply angular | 20.0 |
| Fruit shape | 1 | Globular | 20.0 |
| | 2 | Flattened | 25.0 |
| | 3 | Disk-shaped | 10.0 |
| | 5 | Oval | 5.0 |
| | 6 | Acorn | 5.0 |
| | 7 | Pyriform | 25.0 |
| | 14 | Crooked neck | 10.0 |
| Fruit ribs | 0 | Absent | 35.0 |
| | 3 | Superficial Intermediate | 20.0 |
| | 5 | | 45.0 |
| Predominant fruit skin colour at maturity | 2 | Green | 10.0 |
| | 5 | Yellow | 70.0 |
| | 6 | Orange | 20.0 |
| Secondary fruit skin colour | 0 | No secondary skin | 25.0 |
| | 2 | Green | 75.0 |
| Secondary fruit skin colour pattern | 0 | No secondary friut skin | 55.0 |
| | 1 | colour | 30.0 |
| | 3 | Speckled | 15.0 |
| | | Striped | |
| Fruit skin texture | 1 | Smooth | 65.0 |
| | 3 | Finely wrinkled | 5.0 |
| | 4 | Shallowly wavy | 5.0 |
| | | With warts | 25.0 |
| Flesh colour | 1 | White | 25.0 |
| | 3 | Yellow | 60.0 |
| | 4 | Orange | 15.0 |

Principal component analysis (PCA) generalised nine quantitative characters to the three principal components, which explained 80.75% of the total variability. The first component accounted for 48.30% of the total variation, and was mainly defined by fruit factor, leaf width and fruit peduncle traits. The second component accounted for further 17.67% and was correlated with leaf length. Finally, the third component accounted for 14.78% and was associated with plant length (Table 4). Clearly, the characters with high value in the first and second PCs should be considered more important, since they explain nearly half of the total variation.

*Table 3. Correlation matrix among characteristics studied*

| Traits | LW | L | FPL | FPW | FL | FC | FT | NF | FW |
|---|---|---|---|---|---|---|---|---|---|
| LL | 0,77** | -0,02 | 0,12 | 0,14 | 0,85** | 0,48** | 0,41** | -0,37** | 0,44** |
| LW | | -0,05 | 0,26** | 0,48** | 0,69** | 0,33** | 0,50** | -0,41** | 0,28** |
| L | | | 0,34** | 0,21* | 0,05 | -0,06 | -0,21* | 0,25* | 0,23* |
| FPL | | | | 0,67** | 0,16 | 0,61** | 0,53** | -0,28** | 0,52** |
| FPW | | | | | 0,22* | 0,42** | 0,60** | -0,43** | 0,53** |
| FL | | | | | | 0,38** | 0,27** | 0,20* | 0,49** |
| FC | | | | | | | 0,79** | -0,55** | 0,80** |
| FT | | | | | | | | -0,82** | 0,55** |
| NF | | | | | | | | | 0,36** |

*P<0.05.

**P<0.01.

*Table 4. Eigenvalues, proportion of total variability and correlation betwen the original variables and the first three principal components (PCs).*

| Variable | PC1 | PC2 | PC3 |
|---|---|---|---|
| LL | 0,69 | -0,70 | -0,20 |
| LW | 0,71 | -0,47 | -0,13 |
| L | 0,03 | 0,39 | -0,82 |
| FPL | 0,64 | 0,58 | -0,21 |
| FPW | 0,69 | 0,43 | -0,09 |
| FL | 0,63 | -0,59 | -0,39 |
| FC | 0,84 | 0,17 | 0,15 |
| FT | 0,87 | 0,16 | 0,42 |
| NF | -0,69 | -0,01 | -0,54 |
| FW | 0,78 | 0,21 | -0,21 |
| Eigenvalue | 4,83 | 1,77 | 1,48 |
| % Var. | 48,30 | 17,67 | 14,78 |
| % Cum. | 48,30 | 65,97 | 80,75 |

Distribution of cultivars on the PC1 and PC2 plot indicates variability of this research collection (Fig. 1). Starting from the negative to the positive values of PC1, the *C. pepo* varieties exhibited a general increase in the fruit circumference, fruit length, flesh thickness, fruit weight and leaf width, and a decrease in number of fruits per plant. Starting from the negative towards the positive values of PC2, the varieties were characterized by decreasing leaf length.

The PCA provided a simplified classification of the *C. pepo* varieties for collection and breeding. The scatter plot also shows geometrical distances among the varieties in the plot that reflect their similarity in terms of variables measured. Thus, based on this analysis, five groups of related varieties were separated. Group A includes three varieties with a high negative value of PC1 and an intermediate value of PC2. Group B consist of varieties that correspond to an intermediate value of PC1 and positive values of PC2. Eight varieties that have a low to intermediate value of

PC1 and low to intermediate value of PC2 are in group C. Group D includes two varieties with a low negative value of PC1 and high negative value of PC2. Finally, group E consists of two varieties that have positive value of PC1 and high negative value of PC2. For further selection, it is, thus, sufficient to take just one variety from each group, according to preferred colour. Based on the position, a small genetic distance is observed between CP11 and CP 15, CP14 and CP2, CP7, CP1 and CP16, as well as the CP17 and CP18 varieties. Of particular interest are CP5, CP9 and CP10 varieties, which were located in gaps. For example, CP10 is characterized by large fruit size, nice orange colour and warty texture.

High variability of morphological traits is characteristic for the Cucurbitaceae family. According to research of MLADENOVIĆ *et al.* (2012) and MLADENOVIĆ *et al.* (2013) who examined 44 genotypes and inheritance of some qulititative gens of *Lagenaria siceraria* Molina. (Standl.), variability of fruit characteristics was most important. It is interesting that *Cucurbita pepo* also show large variability in fruit quantitative and qualitative traits.

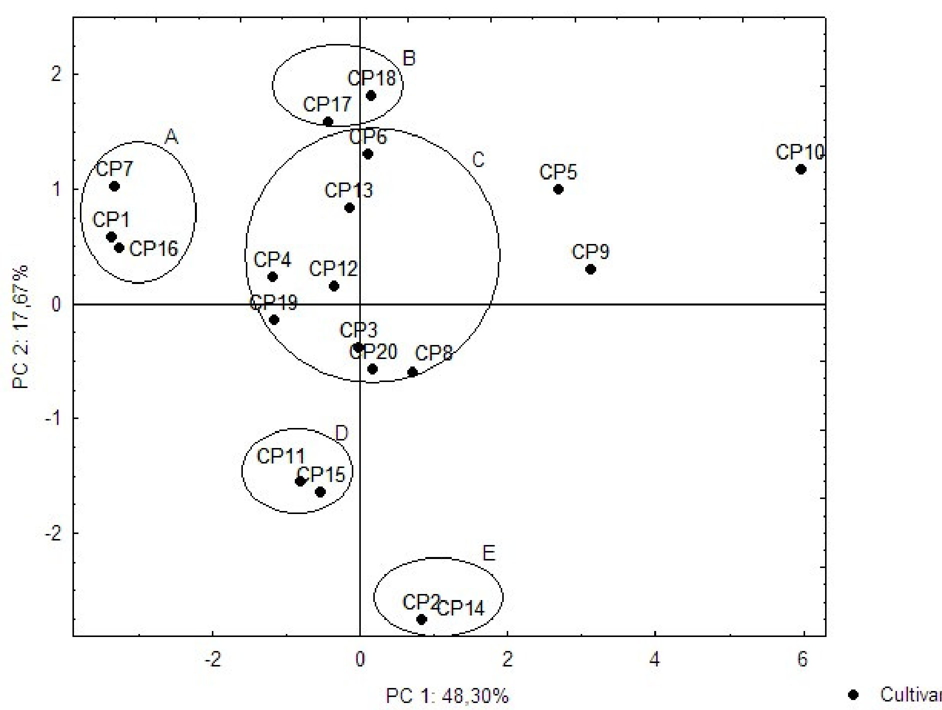


Figure 1. Factor scores for the first two principal components (PCs) for 20 *C. pepo* accessions

## CONCLUSION

The squash varieties differing in the fruit size, colour and texture, leaf and seed size, as well as other traits, were presented in the germplasm studies. Some of the varieties exhibit many favourable fruit characteristics of commercial importance and can thus be a good source of germplasm for breeding. For cultivation and conventional breeding, varieties with high PC1 scores could be good genitors for large fruit size. The great variation of *C. pepo* collection from Serbia is thus confirmed by our findings. The genetic diversity in *C. pepo* is most likely attributed to the genetic variability of the species, suitable for adapting to the diverse agro-ecological conditions. Breeding of squash in future studies will aim to decrease fruit weight, number of seeds per fruit, as well as yield. However, some qualitative traits that enhance decorative value of this species also offer excellent research potential. The rich of diversity provide more selection chances in breeding.

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**GENETIČKI DIVERZITET KOLEKCIJE UKRASNE TIKVE (*Cucurbita pepo* L.)**

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Izvod

Cilj ovog rada je kolekcionisanje i očuvanje jedinstvene germplazme obične tikve (*Cucurbita pepo* L.) u Srbiji. U radu je opisana unutarvrsna varijabilnost 20 genotipova obične tikve. Istraživanje je imalo za cilj da se očuva i ispita reprezentativna kolekcija obične tikve koja će se koristiti u oplemenjivačkom radu. Sakupljen je velik broj genotipova obične tikve. Jedanaest karakteristika je mereno kod 20 genotipova i na taj način determinisan je visok nivo polimorfizma kao i određene sličnosti među genotipovima. Kvalitativne karakteristike koje su pokazale veliku varijabilnost bile su oblik, boja i tekstura ploda. Koeficient varijabilnosti bio je najviši za osobine kao što su dužina ploda, težina ploda i broj plodova po biljci (CV=56.69-161.32%), dok je najniži bio za dužinu lista (CV=20.65%).

Na osnovu PCA rezultata, 20 genotipova obićne tikve grupisani su u pet grupa. Ove grupe imaju jedinstvene kvantitativne i kvalitativne karakteristike. Ovo istraživanje o genetičkoj raznolikostri između genotipova obične tikve je od velike važnosti za dalji oplemenjivački rad na ovoj vrsti.

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