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YIELD AND QUALITY PARAMETERS OF HOKKAIDO TYPE PUMPKINS GROWN IN SERBIA

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The aim of this study was to assess a set of 40 accessions of Hokkaido type pumpkins (*Cucurbita maxima* Duchesne) in terms of the traits of agronomic importance and the basic fruit flesh quality parameters, with the emphasis on Serbian environmental conditions. The pumpkins are a part of the larger *Cucurbita* collection belonging to the Institute of Field and Vegetable Crops from Novi Sad, where the two-year (2016, 2017) field trial was conducted. The accessions significantly differed in all analyzed parameters. The average values for the traits of agronomic importance were: yield per plant 3.56 kg, fruit weight 1.84 kg, number of fruits per plant 2.03, and anthesis date 180.29. Length of the main stem was in most cases short. Among fruit flesh quality parameters; the average dry weight content was 11.49%, sugars content 43.32 mg/g fresh weight, carotenoids content 50.12 mg/kg fresh weight, proteins content 4.04 mg/g fresh weight, total soluble solids content 8.63 °Brix, refractive index 1.347, and pH 6.98. The highest coefficients of variation for agronomic and quality traits were those calculated for yield per plant and carotenoids content. The pumpkins performed better in the first season of the experiment due to more favorable weather, firstly temperature, conditions. The most pronounced adverse effect of high temperatures occurring in 2017 was the one expressed on the content of carotenoids, which was on average 48.4% lower when compared to 2016. Principal component analysis was employed to clarify the relationships among the

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investigated parameters, and to distinguish those with the most important contribution to the pumpkin variability. The analysis revealed strong correlations among yield per plant, fruit weight and length of the main stem. Carotenoids content and pH also correlated to yield, while the other quality parameters correlated to each other. Defining both first and second principal components, yield per plant and fruit weight were the parameters with the highest contribution to the variability among the assessed pumpkins. Concerning quality parameters, fruit flesh dry weight contributed the most to the variability. Therefore, the strategy for breeding Hokkaido type pumpkins adapted to Serbian environmental conditions should be based on combining irrespectively selected high-yield and high-quality accessions.

Keywords: Cucurbita maxima, Hokkaido pumpkin, PCA, quality parameters, yield

INTRODUCTION

Amongst the species of the divergent genus *Cucurbita* L. (pumpkin, squash, gourd), *C. maxima* Duchesne, *C. moschata* Duchesne and *C. pepo* L. are of the greatest agro-economic importance. The three are cultivated for food, feed and ornamentation, widely distributed in the world and known for exceptional polymorphism of fruit shape, colour and size. At the moment, the average yearly production amounts about 3.38 and 23.70 million tons in Europe and in the World, respectively; representing an increase of approximately 66 and 100% when compared to the last decade of the previous century. At the global level and for the same time period, the increased total production is primarily an account of the increased area harvested (78%) and then of the increased yield (13%). Vice versa, the increase in the European pumpkin production is due to the enhanced yield (37%) and enlarged crop area (24%). The current average yield of the pumpkin, squash and gourd is 13.17 and 23.08 tons/ha at the global and European level, respectively. The crops occupy about 1.8 million ha in the World, of which 0.15 million ha in Europe (WU *et al.*, 2007; BERENJI and SIKORA, 2011; MARTÍNEZ-VALDIVIESO *et al.*, 2015a; MARTINS *et al.*, 2015; FAO, 2018).

Both pumpkin fruit seeds and flesh are of valuable dietary quality; the seeds are an excellent source of oils, proteins and minerals, whereas the flesh contains carotenoids, carbohydrates, flavonoids, minerals, amino acids and phenolic compounds. The flesh is of exceptionally low caloric value (approximately 20 kcal/100 g). As in other agricultural plants, pumpkin seed and flesh yield and quality parameters depend on cultivar and environmental conditions during the growth and fruit maturation (AZEVEDO-MELEIRO and RODRIGUEZ-AMAYA, 2007; NAWIRSKA-OLSZAŃSKA *et al.*, 2014; MARTÍNEZ-VALDIVIESO *et al.*, 2015b; PONKA *et al.*, 2015; SOLTANI *et al.*, 2017; MERU *et al.*, 2018).

Based on fruit shape, size, colour and other plant characteristics, numerous types are distinguished within each *Cucurbita* species. In the diverse *Cucurbita maxima* species there are both small orange red kuris weighting about 0.1-0.2 kg and giant pumpkins often exceeding 500 kg, with a multitude of diverse landraces, heirlooms, cultivars and hybrids. There are 113 registered varieties in the European database at the moment. Inconsistent to the mentioned diversity, generally positive attitude of consumers towards pumpkins, as well as to the increasing global and European pumpkin production, there are only four registered varieties in the Serbian database (EUROPEAN COMMISSION, 2018; MINISTRY OF AGRICULTURE, FORESTRY AND WATER ECONOMY, 2018).

Therefore, it would be useful to work on breeding specific types of pumpkins that are adapted to Serbian environmental conditions. One of the types with growing popularity is Hokkaido, an early maturing small type (mostly up to 2.5-3.0 kg), usually of bright orange smooth surface and flesh, transverse elliptic or pear shaped, with thin edible skin, suitable for various culinary uses. Although breeding work on this pumpkin type started at the Institute of Field and Vegetable Crops more than a decade ago, there is no registered variety.

This study was undertaken to assess the basic yield and quality parameters in the collection of Hokkaido pumpkins grown in Serbian agro-ecological conditions, and to examine the interrelationships among these parameters. The second aim was to distinguish the accessions with good performances that will be used for breeding.

MATERIALS AND METHODS

The complete randomized block design trial was set up at the experimental field Bački Petrovac (N45°20', E19°40', 82 m altitude), Institute of Field and Vegetable Crops, Novi Sad, Serbia. The trial included 40 accessions of Hokkaido type pumpkins (*Cucurbita maxima* Duchesne), selected mostly on the basis of fruit and the whole plant characteristics from a larger collection of lines developed in breeding programs, and designated as Hokk1, Hokk2, etc. The plants were grown in two consecutive years (2016, 2017). The sowing was performed in the first decade of May, and the fruit harvest in the second half of September. The main plot consisted of a row with five 1.5 m spaced plants, and it was replicated three times. Since a part of the collection grows in a vining manner, the spacing between rows was 5.0 m. The agro-technical procedures, mostly related to the mechanical weed control, were performed according to usual pumpkin production practice. When needed, the plants were irrigated. Significant diseases were not recorded, however; the exceptionally delicious thin-skinned fruits attracted the rabbits, and therefore the plots had to be fenced. The weather data for the pumpkin growing seasons, officially reported by the REPUBLIC HYDROMETEOROLOGICAL SERVICE OF SERBIA (2018), are given in Table 1.

The following traits of agronomic importance were analyzed: yield per plant (kg), fruit weight (kg), number of fruits per plant, anthesis day (number of days from January 1st to anthesis) and length of main stem (1-very short, 3-short, 5-medium, 7-long, 9-very long); whereas the chemical analyses of fruit flesh included the content of dry weight (%), sugars (mg/g fresh weight), proteins (mg/g fresh weight) and carotenoids (mg/kg fresh weight), pH, refractive index and total soluble solids (°Brix). The parameters total soluble solids content and refractive index were determined by Abbe refractometer, and carotenoids content according to the method described by WELLBURN (1994). Fresh plant material (0.2 g) was ground with cooled mortar and pestle and homogenized with 80% acetone. The extracts were centrifuged for 10 min at 4350xg. Absorbance was recorded at 470, 646 and 663 nm. Carotenoids content was calculated from equations described in the applied method. For aqueous extracts, 10.0 g of fresh plant material was homogenized and 30.0 ml of distilled water was added. After 30 min shaking, extracts were centrifuged for 10 min at 4350xg and tested for pH, proteins and sugars contents. Proteins content was determined by BRADFORD method (1976). Total carbohydrate content (only sugars) was estimated according to the slightly modified sulfuric acid-UV method, reported by ALBALASMEH *et al*. (2013). Aqueous extract (1.0 ml) was rapidly mixed with 3.0 ml of concentrated sulphuric acid, vortexed 30 s and cooled in ice for 2 min (due to a rapid temperature rise after addition of sulphuric acid). Finally, UV light absorption was read at 315

nm. Appropriate dilution with distilled water was applied to obtain absorbance between 0.200 and 0.800. Reference solutions (glucose) were prepared following the same procedure as cited above. Total sugar content was expressed as mg glucose equivalents per kg fresh weight. Statistical analysis of data, which comprised basic statistics, factorial analysis of variance and principal component analysis, was performed using Statistica 12 (StatSoft, Dell Inc., Tulsa, USA; University License, Novi Sad) software package.

Table 1. Temperature and precipitation parameters for Hokkaido type pumpkin growing seasons (May-September) of 2016 and 2017, with long-time averages

		Month				
Parameter	Year	May	June	July	August	Sept.
Average daily temperature (°C)	2016	16.9	21.7	22.8	21.1	18.5
	2017	17.5	22.9	23.9	24.7	17.9
	1981-2010	17.3	20.1	21.9	21.6	16.9
Sum of temperatures (°C)	2016	523.9	651.0	706.8	654.1	555.0
	2017	542.5	687.0	740.9	765.7	537.0
	1981-2010	536.3	603.0	678.9	669.6	507.0
Maximum monthly temperature	2016	30.8	34.8	34.3	33.6	31.9
(°C)	2017	30.2	36.6	37.8	39.8	33.5
	1981-2010	34.0	37.6	41.6	40.0	37.4
Number of days with maximum	2016	1.0	6.0	13.0	5.0	8.0
daily temperature above 30.0 °C	2017	1.0	11.0	18.0	22.0	4.0
	1981-2010	1.4	6.3	11.2	11.5	2.0
Number of days with maximum	2016	0.0	0.0	0.0	0.0	0.0
daily temperature above 35.0 °C	2017	0.0	2.0	6.0	13.0	0.0
	1981-2010	0.0	0.5	1.0	1.7	0.1
Sum of precipitation (mm)	2016	85.0	143.2	68.4	45.8	33.7
	2017	82.8	66.8	12.3	17.0	83.0
	1981-2010	63.0	91.4	64.3	57.5	53.8

RESULTS AND DISCUSSION

Significant differences have been found among the analyzed Hokkaido type pumpkins in terms of all the investigated traits (not shown). Since the accessions for this study were assorted on the basis of the diversity of previously surveyed, mainly agronomic traits, the differences were rather expected. The significant differences noted between the two meteorologically dissimilar growing seasons (Table 1, 2), as well as the interactions between the pumpkin accessions and the seasons were also expected. The variability of *Cucurbita maxima*, *moschata* and *pepo* genotypes and their specific responses to different environmental conditions and constraints are well-documented by other authors, e.g. MLADENOVIĆ *et al.*, 2014; CONTI *et al.*, 2015; PARIS, 2016; BAKHTOURI *et al.*, 2017; MISHRA, 2017; ZHAO *et al.*, 2017; MARTÍNEZ *et al.*, 2018.

Considering the traits of agronomic importance, the greatest variation among the pumpkin accessions was observed for yield per plant, which is common for complex traits and in accordance to the results of SULTANA *et al.*, 2015 and CHAUDHARY *et al.*, 2017a. In addition, yield components fruit weight and the number of fruits per plant varied in considerable ranges.

Contrary to the commonly reported significant variability among pumpkins for anthesis date, e.g. AHMED *et al.*, 2017; the trait was with small coefficient of variation in our study. The discrepancy is understandable considering the fact that all the examined accessions were of the same Hokkaido type, which is in Serbian environmental conditions among the earliest-flowering pumpkins. Similar to the study of NAHAR *et al.*, 2016; the pumpkins differed in terms of the length of main stem, although the Hokkaido type pumpkins are in most cases bushy.

Table 2. Basic statistic parameters (means, ranges, coefficients of variation – CV, %) for yield and quality parameters of 40 Hokkaido type pumpkin accessions grown in two seasons

Parameter	2016			2017		
	Mean	Range	CV	Mean	Range	CV
Yield per plant (kg)	3.90^{a}	0.80-20.52	89.35	3.22^{b}	1.06-12.82	75.57
Fruit weight (kg)	1.91^{a}	0.57-7.60	70.30	1.78^{b}	0.31-6.14	61.68
Number of fruits per plant	2.13^{a}	1.00-8.50	54.46	1.94^{b}	1.00-9.00	64.44
Anthesis date	184.4^{a}	182.0-185.0	0.43	176.2^{b}	171.0-191.0	1.86
Length of main stem	3.0^{a}	1.0-9.0	98.5	3.0^{a}	1.0-9.0	98.5
Dry weight (%)	11.30^{a}	5.20-18.47	26.33	11.67^{a}	7.78-19.80	20.20
Sugars (mg/g fresh weight)	41.19^{a}	13.28-80.91	34.43	45.45^{b}	5.34-96.70	50.80
Carotenoids (mg/kg f.w.)	66.12^{a}	0.01-138.72	52.88	34.11^{b}	1.39-85.99	52.15
Proteins (mg/g f.w.)	4.21^{a}	2.32-6.19	22.48	3.88^{b}	2.01-6.11	24.61
Refractive index	1.345^{a}	1.334-1.354	0.27	1.349^{a}	1.341-1.470	1.45
Total soluble solids (°Brix)	8.39^{a}	4.0-14.0	26.86	8.88^{b}	5.50-12.50	18.33
pH	7.01^{a}	4.07-7.41	7.16	6.95^{a}	6.56-9.9.96	7.32

^{a,b}Values within the rows followed by the same letter do not differ at the 0.05 level of probability, according to LSD test

The values and the variation of the parameters related to fruit flesh quality were substantially within the range of the published values (MURKOVIC *et al.*, 2002; JACOBO-VALENZUELA *et al.*, 2011; KIM *et al.*, 2012; CHAUDHARY *et al.*, 2017b). The content of the carotenoids and sugars in fresh pumpkin flesh were with the highest variation; implying the possibility for breeding pumpkins with improved values of these important compounds. Although the analyses of sugars are traditionally related to both total soluble solids content and refractive index (DINU *et al.*, 2016; ZHOU *et al.*, 2017), the almost negligible differences among the studied accessions in terms of the refractive index indicate total soluble solids as a more usable parameter for further research on pumpkin fruit flesh quality.

Nevertheless, the values of the majority of the analyzed traits differed between the two growing seasons. Among the traits of agronomic importance, the only exception was the length of the main stem, which was expressed as a descriptive characteristic where the estimates remained equal in both seasons, for all the studied accessions. Considering yield per plant and yield components fruit weight and the number of fruits per plant, the accessions performed better in the first season of the experiment due to more favorable weather, firstly temperature, conditions. As for the season of 2017, average daily temperatures and sums of temperatures were mainly higher when compared to 2016 and long-term averages. However, the fertilization, formation and development of the fruits were particularly adversely affected by high (> 30.0°C) and extremely high (> 35.0°C) maximum daily temperatures, occurring especially in August.

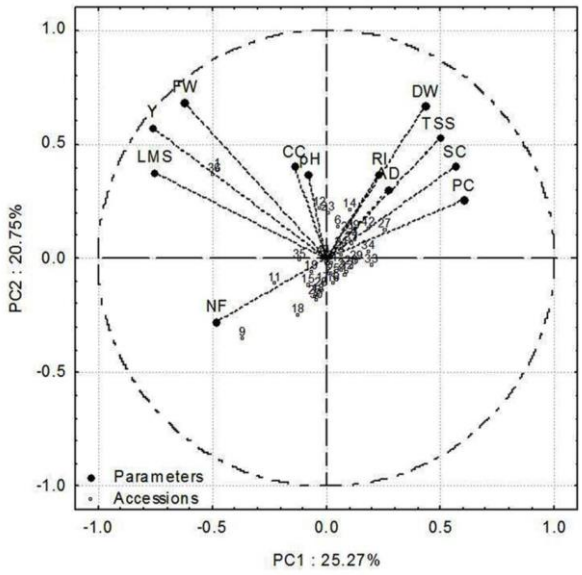
Since the insufficient precipitation was compensated by irrigation, we assume that the high temperatures were the main cause of the comparatively low pumpkin yields recorded in 2017. As for the traits related to fruit flesh quality, the most pronounced adverse effect of high temperatures was the one expressed on the content of carotenoids, which was on average almost halved in 2017 comparing to the more favorable 2016 season. Out of 40 analyzed accessions, only two were with slightly higher carotenoids content in the generally less favorable season (not shown). The adverse effect of even moderately higher external temperatures (> 25.0°C) on carrot carotenoids content was documented by LESTER (2006). Concerning other quality parameters; sugars and soluble solids contents were moderately higher in the conditions of temperature stress, proteins moderately lower; while other traits remained with similar values over the two growing seasons.

Table 3. Principal component scores for the analyzed Hokkaido type pumpkin fruit yield and quality parameters

	Main component				
Parameter	PC1	PC2	PC3	PC4	PC5
Yield per plant	-0.760	0.573	0.148	0.130	-0.081
Fruit weight	-0.622	0.686	0.153	0.066	-0.265
Number of fruits per plant	-0.484	-0.275	-0.242	0.096	0.702
Anthesis date	0.272	0.303	-0.651	0.152	-0.023
Length of main stem	-0.751	0.375	-0.098	0.347	0.111
Dry weight	0.433	0.666	0.313	-0.028	0.260
Sugars	0.567	0.402	0.324	0.074	-0.014
Carotenoids	-0.141	0.401	-0.254	-0.601	0.243
Proteins	0.601	0.256	-0.362	0.242	-0.105
Refractive index	0.225	0.370	-0.631	0.277	0.004
Total soluble solids	0.496	0.528	0.346	0.060	0.369
pH	-0.083	0.369	-0.314	-0.638	-0.128
Eigenvalue	3.03	2.49	1.55	1.09	0.87
Total variance (%)	25.26	20.75	12.94	9.06	7.27
Cumulative eigenvalue	3.03	5.52	7.07	8.16	9.03
Cumulative variance (%)	25.27	46.02	58.96	68.02	75.29

In order to clarify the relationships among the investigated yield and quality parameters, and to distinguish the most important parameters contributing to the observed variability of the pumpkin accessions, the principal component analysis was employed (Table 3). Two-year mean values were used for the analysis. The number of principal components considered for further analysis is usually determined according to eigenvalues that are > 1.00 (ETICHA *et al.*, 2010; BOSHEV *et al.*, 2016), thus in our investigation the first four components which contributed with 68.02% to total variance would be sufficient. Since the ratio of the explained variance rapidly declines from the third component, the attention was primarily paid to the first and second component which together explained 46.02% of the total variance. The most important parameters in the first component (25.27% of total variance) were yield per plant, length of the main stem, fruit weight and proteins content; while fruit weight, dry weight of fruit flesh, yield

per plant and total soluble solids content dominated in the second (20.75%) principal component. Yield per plant and fruit weight defined both components and therefore represent the parameters with the highest contribution to the variability among the assessed pumpkins. On the contrary, the results of SULTANA *et al.* (2015) imply the number of fruits per plant as an important component of variability in a set of studied *Cucurbita moschata* accessions. Although belonging to the same family, *C. moschata* and *C. maxima* are different plant species characterized by numerous agronomic types. Obviously, the set of Hokkaido type pumpkins that was the subject of our investigation is more variable in terms of the fruit weight than in terms of the number of fruits per plant. Nevertheless, the traits of agronomic importance were more important for explaining the total variance than the traits related to fruit flesh quality. The scores of the quality parameters were similar to the results of PEVICHAROVA and VELKOV (2017) and imply dry weight as the most contributing to the total variance.



Parameters: Y – yield per plant, FW – fruit weight, NF – number of fruits per plant, AD –anthesis date, LMS – length of main stem, DW – fruit flesh dry weight, SC – sugars content, CC – carotenoids content, PC – proteins content, RI – refractive index, TSS – total soluble solids, pH – pH Accessions: 1 – Hokk1, 2 – Hokk2 etc.

Figure 1. Biplot of PC1 and PC2

The plot of the first and second principal component (Figure 1) shows the strong correlations among yield per plant, fruit weight and length of the main stem. Out of seven studied parameters related to fruit flesh quality, only the content of carotenoids and pH correlated to yield. We assume this was at least partially related to the previously mentioned pronounced environmental, especially temperature effects on yield and carotenoids content. The other quality parameters correlated to each other. Therefore, the strategy for breeding Hokkaido

type pumpkins adapted to Serbian environmental conditions should be based on combining irrespectively selected high-yield and high-quality accessions.

As depicted, the majority of the accessions are grouped close to the biplot origin. The distribution is such because the plant material used in the study consisted of the pumpkins of the same Hokkaido type which are a priori similar. Nevertheless, the employed PCA method distinguished Hokk1 and Hokk36 as the accessions with a good potential regards the traits of agronomic importance, as well as Hokk14, Hokk27 and Hokk42 concerning fruit flesh quality. The accessions Hokk12 and Hokk23 should be considered when breeding for the improved carotenoids.

CONCLUSIONS

Significant differences were noted among the assessed Hokkaido type pumpkin accessions and between the two years of the experiment in terms of all the analyzed traits of agronomic importance and traits related to fruit flesh quality.

High (> 30°C) and extremely high (> 35.0°C) temperatures occurring in the second year of the experiment exhibited adverse effects on the pumpkins. The most affected was carotenoids content which was almost halved with respect to the more favorable year.

Yield per plant, fruit weight and length of the main stem correlated. Carotenoids content and pH also correlated to yield. The other quality parameters correlated to each other.

Yield per plant and fruit weight were the parameters with the highest contribution to the variability among the assessed pumpkins. Concerning quality parameters, fruit flesh dry weight contributed the most to the variability.

Breeding Hokkaido type pumpkins attended for field production in Serbian environments should be based on crosses between irrespectively selected high-yield and high-quality accessions.

In addition, further research on the optimization of agro-technical procedures for the production of this type of pumpkins is required in order to maximize their potential for yield and quality.

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PARAMETRI PRINOSA I KVALITETA BUNDEVA HOKAIDO TIPA GAJENIH U SRBIJI

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Izvod

Cilj rada je ocena 40 akcesija bundeva Hokaido tipa (*Cucurbita maxima* Duchesne) u pogledu svojstava od agronomskog značaja i parametara kvaliteta mesa ploda, sa naglaskom na agroekološke uslove Srbije. Bundeve pripadaju većoj *Cucurbita* kolekciji Instituta za ratarstvo i povrtarstvo, Novi Sad, gde je izveden dvogodišnji (2016, 2017) poljski ogled. Akcesije su se značajno razlikovale u pogledu svih parametara. Srednje vrednosti agronomskih svojstava su: prinos po biljci 3,56 kg, masa ploda 1,84 kg, broj plodova po biljci 2,03, datum cvetanja 180,29, kratka glavna stabljika. Kod parametara kvaliteta; sadržaj suve materije je 11,49%, šećera 43,32 mg/g sveže mase, karotenoida 50,12 mg/kg, proteina 4,04 mg/g, ukupnih rastvorljivih čestica 8,63°Brix, refrakcioni indeks 1,347, pH 6,98. Najviši koeficijenti varijacije izračunati su za prinos po biljci i sadržaj karotenoida. Utvrđeni su veći prinos i bolji kvalitet u prvoj godini istraživanja. Nepovoljni efekat visokih temparatura u 2017. odrazio se najviše na sadržaj karotenoida, koji je bio 48,4% niži nego u 2016. Da bi se istražili odnosi parametara, kao i radi izdvajanja parametara sa najvećim doprinosom varijabilnosti bundeva, primenjena je analiza glavnih komponenata. Uočene su jake pozitivne korelacije prinosa po biljci, mase ploda i dužine glavne stabljike. Sadržaj karotenoida i pH su korelirali sa prinosom, dok su ostali parametri kvaliteta korelirali međusobno. Prinos po biljci i masa ploda su najviše doprineli varijabilnosti istraživanih bundeva. Kod parametara kvaliteta, varijabilnosti je najviše doprinela suva masa mesa ploda. Strategija za oplemenjivanje bundeva Hokaido tipa prilagođenih uslovima sredine Srbije bi trebala biti zasnovana na kombinovanju nezavisno selekcionisanih visokoprinosnih i visokokvalitetnih akcesija.

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