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Research Article

Breeding potential of morphological and phytochemical characteristics of landraces and autochthone varieties of *Capsicum annuum* L. in Republic of Serbia

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Abstract

By maintaining a large number of local pepper genotypes, it is possible to preserve a large number of desirable genes that can be used in different pepper selection programs. The aim of this study was to classify a part of the collection of 15 genotypes (11 populations and 4 varieties originating from autochthonous populations) of peppers originating from Republic of Serbia. Morphological characteristics (weight, length, width, pericarp thickness, color before fruit ripening, shape and number of fruit chambers) and 10 phytochemical parameters of the fruit (carbohydrates, essential oils, ash, cellulose, beta carotene, potassium, iron, vitamin C, total phenols, antioxidant activity) were studied. Using statistical multivariate techniques (PCA and Cluster analysis), the degree of variation between local populations was assessed and diversity was determined based on the morphological and nutritional characteristics of pepper fruits. Morphological traits were determined using two main components that accounted for 70.3% of the variability. These components accounted for 49.8% of the variation in nutritional traits. The 'Čokotanka' population would be suitable for individual selection and reduced divergence within the population, since it has 6 tested traits that resulted in high diversity index. Populations 'Stojankina kletva', 'Lalić' and 'Strižanka' would be suitable for recombination of genes to improve the properties of individual selection programs for this vegetable species.

Keywords: cluster analysis; landraces; pepper; PCA; Shannon-Weaver Diversity Index

Introduction

The genus *Capsicum* includes a large number of wild and cultivated species, the fruits of which are grown worldwide. Fruits are an excellent source of health- beneficial ingredients, such as ascorbic acid (vitamin C), carotenoids (provitamin A), tocopherol (vitamin E), flavonoids and capsaicinoids. Pepper fruits are used fresh and thermally processed, as well as for medicinal purposes, such as the treatment of asthma, cough, sore throat

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and toothache (Wahyuni *et al.*, 2013). The genus *Capsicum* (sweet and hot peppers) shows intra and inter-specific diversity in fruit type, color, shape, taste and biochemical content (Dagnoko *et al.*, 2013). Different forms of fruits arise as a result of the development and differentiation of the gynoecium after fertilization. They are products of late morphological and structural modifications in carpels (Cutler *et al.*, 2008). The characteristic morphological features of fruits have systematic value and represent an important taxonomic tool at the levels of family, genus, species, and variety, since they differ in different taxa (Zhigila *et al.*, 2014). Breeding the pepper according to morphological characteristics is caused by traditional needs in food preparation, which causes selection that leads to a reduction in low-frequency alleles in the pepper population, which in the long term can cause significant genetic erosion of the landrace of the pepper population (Portis *et al.*, 2004). Genotype × environment interaction testing is used to identify genotypes that are best suited to specific environmental conditions and to provide the best approach for selection to maximize productivity (Anilkumar *et al.*, 2021).

The variability and genetic diversity of pepper are wide, which allows for very good gene recombination. Biodiversity protection has become a top priority worldwide because of the world as the emerging need to protect genetic resources closely related to adaptation to climate change. Drastic reduction in the genetic diversity of cultivated pepper (*Capsicum annumm* L.) requires the conservation and use of all existing genetic resources (Pickersgill, 1997; Do Rego *et al.*, 2003; Geleta *et al.*, 2005; Barroso *et al.*, 2012; Dagnoko *et al.*, 2013; Danojević and Medić-Pap, 2018.).

Pepper has a high vitamin C content (higher than other vegetables) and is a significant source of this vitamin in the human diet (Lee *et al.*, 2000). Many studies have shown that pepper is an excellent reservoir of vitamin C, not only fresh, but also after heat treatment. The highest soluble solid content was found in pepper fruits at physiological maturity (Valšíková *et al.*, 2017). Non-mature (green) pepper fruits had the highest level of polyphenols, while biologically mature (red) fruits had the highest levels of vitamin C and provitamin A (Marín *et al.*, 2004; Wahyuni *et al.*, 2013).

The interrelationship between the analysed parameters and cultivars showed that ascorbic acid could represent the factor with the greatest influence on antioxidant activity. The polyphenol profile differs among cultivars, with a prevalence of phenols in yellow cultivars and flavonoids in red cultivars (Fratianni *et al.*, 2020). The analysis of the main components of the variability of one population showed that the ripening stage and cultivation system-organic and conventional, influence the phenolic composition and antioxidant capacity of pepper (Guilherme *et al.*, 2020).

One of the primary reasons for preserving plant genetic resources in gene banks the protection of their genetic diversity. The main pepper collections were located in the Russian Federation (2313), Germany (1504), France (1400), and Hungary (1400). A duplicate collection of morphological and physiological markers from the *Capsicum* Genetic Cooperative, including morphological and physiological markers, was stored at the University of Turin (Italy). European collections contain pepper specimens from all over the world, as well as characterized genetic stocks and resources evaluated for disease resistance genes and fruit characteristics as the most divergent traits in peppers (Maggioni, 2004; Occhiuto *et al.*, 2014). The more diverse the initial collection, the more successful the breeder's work (Danojević and Medić-Pap, 2018).

Systematic collection of old and local pepper populations and the preservation of their phenotypic diversity can be utilized in future breeding programs for variety improvement (Aklilu *et al.*, 2016; Sahin *et al.*, 2022). The foundation of all high-yielding varieties lies in genetic resources resistant to biotic and abiotic stress conditions to ensure the sustainability of plant production and environmental protection (Sahin *et al.*, 2022). Information about local diversity is essential for preserving variability, avoiding genetic erosion, and ensuring food security (Santos *et al.*, 2019). It is estimated that about 20% of global biodiversity is lost due to continuous human misuse of resources and pollution caused by human activities (Özhatay *et al.*, 2009).

By preserving a greater number of local genotypes, the possibility of preserving a greater number of desirable genes, which can be used in various pepper selection programs, can be increased. The assumption is that by applying multivariate techniques, the degree of variation within one population can be assessed and the diversity between local pepper varieties can be determined. The ultimate goal was to recognize desirable genotypes and include them in the selection program. Morphological and nutritional characteristics, of this economically important vegetable.

Materials and Methods

Morphological characterization

The material used in this research were 15 pepper genotypes: 11 local populations and 4 varieties originating from local populations. Original genetic material collected from all parts of Republic of Serbia was used for testing (Table 1). Pepper fruits were mostly classified according to their different nutritional uses.

Pepper was grown according to the standard method of mid-early production in the open field. The experiment was set up in the random block system with three repetitions, on experimental plots of 1 × 3 m, at an inter-row distance of 50 × 25 cm between plants in a row.

Three fruits from each plant, picked by hand at the botanical stage of maturity were analysed. The number of harvests ranged from two to three, until three fruits at physiological maturity were harvested from each plant.

The following traits were determined in 10 plants: fruit weight (**W**) in grams, fruit length (**FL**), fruit diameter (**FD**) and pericarp thickness (**PT**) in cm, color of fruit before maturity (**CFBM)**, shape of longitudinal section of the fruit (**SLSF),** prevailing number of fruit chambers (**PNFCH**), according to the IPGRI descriptor (1995). Fruit shape index (**FSI**) was calculated by dividing the fruit length by the diameter at the middle of the fruit (FL/FD). Both morphological and phytochemical parameters of the fruits were examined at physiological maturity.

Table 1. Pepper genotypes used in the experiment, their sources, and fruiting habit traits

Genotypes	Fruit shape	Color of ripe fruit	Origin	Coordinates	
				N (North)	S (South)
'Ruska žuta'	square	yellow	Rusko selo (Kikinda)	N: 45°45′28″	E: 20°34′11″
'Čokotanka'	cordate	red	Laplje selo (Kosovo and Metohija)	N: 42°59′40″	E: 21°13′64″
'Kurtovska kapija'	cordate	red	Kosovska Mitrovica	N: 42°88′35″	E: 20°85′68″
'Žabara'	cordate	red	Leposavić	N: 43°10′96″	E: 20°80′08″
'Somborka'*	square	red	Sombor	N: 45°81′83″	E: 19°17′30″
'Bobinka'	circular	red	Pirot	N: 43°13′61″	E: 22°59′85″
'Vrtka'	cordate	red	Bela Palanka	N: 43°22′76″	E: 22°32′70″
'Banana'	Horn-shaped	orange	Lozovik (Velika Plana)	N: 44°47′59″	E: 21°06′05″
'Rošajka'	Horn-shaped	red	Zavoj (Pirot)	N: 43°27′79″	E: 22°64′18″
'Stojankina kletva'	narrowly triangular	yellow	Markovac (Velika Plana)	N: 44°23′80″	E: 21°13′59″
'Lalić'	cordate	red	Lalić (Odžaci)	N: 45°51′41″	E: 19°38′56″
'Kolonija'*	moderately triangular	red	Vranje	N: 42°53′63″	E: 21°88′14″
'Sivrija'	moderately triangular	red	Pepeljevac (Kruševac)	N: 43°56′68″	E: 21°25′45″

'Krivopetlja'	moderately triangular	red	Lipnički šor (Loznica)	N: 44°58′46″	E: 19°26′13″
'Strižanka'*	moderately triangular	red	Striža (Paraćin)	N: 43°83′91″	E: 21°41′07″

*Genotypes are varieties that are in the Register of Recognized Varieties of the Republic of Serbia

Ten (10) phytochemical parameters of the fruit were determined (carbohydrates-CH, essential oils-EO, ash-ACH, cellulose-CEL, beta carotene-CAR, potassium-K, iron-Fe, vitamin C-vit C, total phenols-TF, antioxidant activity-AA) with ten plants per plot. All experiments were performed in triplicate, and the values were expressed as mean, and coefficient of variation (%).

Beta (β)-carotene was determined according to the method described by Nagata and Yamashita, 1992. The dried ethanolic extract (100 mg) was mixed with 10 mL of an acetone-hexane mixture (4:6) and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. The β-carotene content was calculated according to the following equation:

$$\beta\text{-Carotene (mg/100 mL)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$$

Total phenolics were determined in ethanolic extracts (20 g of samples in 100 cm3 of ethanol according to the Folin-Ciocalteu method (Singleton et al 1999). Gallic acid (GA) was used to calculate the standard curve. Tests were carried out in triplicate; results were mean values ± standard deviations and expressed as mg of gallic acid equivalents per gram of dry extract (mg GA g^{-1}).

Determination of vitamin C (ascorbic acid - Tillmans method): 100 cm^3 of juice was extracted from the sample by pressing, mixed with an equal volume of a mixture of HPO_3 and CH_3COOH solutions and filtered. 10 cm^3 of the filtered sample (containing 5 cm^3 of juice and 5 cm^3 of HPO_3 and CH_3COOH) was measured with a pipette and transferred to Erlenmeyer flasks. Each sample was titrated with Tillmans Reagent (TR) solution until a pale pink color persisted for approximately 5 seconds. At the same time, the blank sample was also titrated with the TR solution to a pale pink color (Cvijović and Aćamović-Đoković, 2005).

The Bertrand method can determine all mono- and disaccharides (with a free hemiacetal group) that reduce metal ions (Cu^{2+} u Cu^{+}), and are oxidized to the corresponding carboxylic acid. The amount of copper (I) oxide formed is equivalent to the amount of the reducing sugar. The Bertrand III solution dissolves the precipitate of copper (I)-oxide, whereby copper (I) changes back to copper (II), and iron (III) is reduced to iron (II). In an acidic environment, the formed iron (II) ions are oxidized with an equivalent amount of potassium permanganate to iron (III), and manganese (VII) changes to manganese (II) (Cvijović and Aćamović-Đoković, 2005).

Cellulose measurement procedure: 10.0 g of sample is quantitatively transferred to an Erlenmeyer flask with a ground neck, poured with 25 cm^3 of cellulose reagent, connected to a return condenser and boiled for 30 minutes on an electric heater over an asbestos mesh. After cooking, the hot content was filtered through a filter paper, dried to a constant mass and weighed. The difference in the mass of filter the paper with and without sediment is the mass of cellulose in the sample (Cvijović and Aćamović-Đoković, 2005).

Determination of total ash: the plant sample was burned directly at a temperature of 500-550 °C to prevent loss of content. An amount of the average sample was accurately measured in a preheated incineration vessel ± 0.001 g and burnt. After firing was complete, the containers with the sample were transferred to the annealing furnace. Combustion lasts 3-4 hours, until a homogeneous mass was obtained. The process of burning, cooling in a desiccator and measurement was repeated until a constant mass of ash was obtained (Cvijović and Aćamović-Đoković, 2005).

Essential oils were isolated from plant materials by distillation. Water vapor was passed through the plant material contained in the distillation flask. The cells of the plant material that contain an essential oil that bursts and evaporates. The mixture of water vapor and essential oil was considered and collected in drops in the receiving vessel. Due to differences in relative density, the essential oil separates from the water, and is separated into a separatory funnel (Cvijović and Aćamović-Đoković, 2005).

The ground plant samples, weighed at 2 g (accurate to four decimal places) were left to stand for 24 hours in 100 ml wide-mouthed Erlenmeyer flasks, to which 30 ml of HNO_3 was added. The samples were then evaporated to a small volume and cooled, filtered into normal 50 mL vessels, made up with deionized water and stored in PVC vials until quantitative spectrometric determination of ICP. iTEVA operating software for the series iCAP 6,000 enables control of all instrument functions (Velimirović, 2013).

Total antioxidative (AO) activity was determined spectrometrically at 517 nm DPPH (1.1-Difenil-2-pikrilhidrazil) (Xu *et al.,* 2010). The ability to neutralize 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in pepper extracts was determined by the spectrophotometric method according to Espin (Espin *et al.*, 2000). The method is based on monitoring the color change of the solution of the DPPH• radical (colored purple) to its reduced form (DPPHH), which is colored yellow. The antioxidant activity results for the liquid extracts were expressed as IC50 (mg mL^{-1}), which represents the concentration leading to neutralization 50% DPPH of radicals in the reaction mixture.

Diversity index

Shannon-Weaver diversity index (H') was computed for each character from the frequency distributions observed in different classes as follows:

$H' = 1 - \sum (i-1) \wedge n\ p_i \log_e p_i$

Where, H' = Shannon-Weaver Diversity Index;

pi = the proportion of genotypes in the i-th class of an n–class character;

n= the number of phenotypic trait classes.

The phenotypic diversity estimates between autochthonous pepper populations were made following the methods described by Wachira *et al.* (1995). Each diversity index value was divided by its maximum value ($\log_e^n$) and normalized to keep the values between 0 and 1, with what it is 0.1≤0.4 mli diversity, 0.4≤0.6 mean and 0.6≤1 high diversity (Eticha *et al.,* 2005).

PCA and cluster analysis

Principal component analysis was used to determine the source and structure of variation as well as their contribution to overall trait variability. The main components were extracted until Eigen value was >1. For Ward's cluster, Euclidean distance squared analysis was used to construct the dendrogram. Divergence was estimated using the Euclidean distance with attachment of complete genes' to grouping. Statistical analyses were performed using STATISTICA 10 (StatSoft, Inc., Tulsa, OK, USA).

Results and Discussion

The basic morphological parameters of the collection of 11 diverse local and 4 varieties originating from local populations from Republic of Serbia were examined in this study and presented in a Table 2. The result indicates that the collection of local populations represents diverse genetic material of autochthonous pepper varieties, which is still in production in the Republic of Serbia and is used for different ways in daily diet of the population. The average values very implicatively show that the examined genotypes belong to different types that were formed according to the way of use. The weight of the fruits is in the range from 8.4 g ('Stojankina kletva' ('S. kletva')) to 183.4 g ('Ruska žuta'). The descriptive morphological parameters are somewhat different, where these characteristics indicate better phenotypic uniformity and belonging to a particular type of edible fruit. For the stability of the population, it is safer to rely on CV% which indicates the percentage of variation within the sample and can indicate the genotypic uniformity of the population. Thus, the coefficient of variation for fruit weight was in the range from 7.9% ('Vrtka') to 31.8% ('Kolonija'). A low percentage of the coefficient of variation (CV) indicates that the 'Vrtka' population is at a high level of genetic purity. It is similar with the parameters that are evaluated descriptively. For the trait the shape on the longitudinal section of the fruit (SLSF percentage of the coefficient of variation was in the range from 3.9% ('Sivrija') to 44.9% ('Vrtka and Lalić'), based on the analysis of results shown in Table 2.

The obtained results are in agreement with most of the results of other researchers who determined the high variability of pepper fruit characteristics, both the average value and the coefficient of variation (Fonseca *et al.,* 2008; Ilić *et al.,* 2013; Danojević and Medić-Pap, 2018). Lahbib *et al.* (2012) conducted an analysis that showed that both yield per plant and placenta weight were positively correlated with the number of fruits per plant, while fruit diameter and fruit pericarp thickness were negatively correlated with fruit length.

The obtained results of the population in the phenotypic sense were more closely explained by the index of diversity (H′) which was determined within one genotype. It was pointless to express the diversity index between individual genotypes, because the values would be high considering that the collected genetic material belongs to different types of pepper fruit. The results show that a high diversity index was determined for fruit mass for all studied populations individually, so it is possible to carry out individual selection within this trait and increase the homogeneity of the population. For the trait of fruit length, a low diversity index was determined only in the genotype 'Sivrija' (0.27). It was high in other populations. For the fruit width trait, the genotype 'Rošajka' had a low (0.39) and 'Lalić' (0.41), while the others had high diversity index.

Table 2. Average values and coefficient of variation of morphological traits

Genotypes	Weight W in g	CV%	Length FL/cm	CV%	Width FD/cm	CV%	Pericarp thickness/cm PT	CV%	Color before ripening CFBM	CV%	Shape in longitudinal section SLSF	CV%	Number of chambers PNFCH	CV%	Index of shape
'Ruska žuta'	183.4	18.1	8.6	11.0	6.20	7.8	0.55	15.7	2.80	15.1	4.30	11.2	3.40	15.2	0.72
'Čokotanka'	151.7	16.8	13.6	10.7	6.21	13.6	0.44	21.9	1.60	32.3	5.20	15.2	2.30	21.0	0.50
'Kurtovska kapija'	136.3	8.5	11.5	6.2	4.52	8.4	0.49	15.1	2.90	10.9	4.10	40.6	2.20	19.2	0.39
'Žabara'	72.5	14.9	10.3	31.8	4.03	13.6	0.30	0.0	2.70	17.9	3.90	42.6	2.10	15.1	0.39
'Somborka'	127.6	11.7	8.6	7.3	5.83	12.0	0.53	17.1	1.80	23.4	4.80	32.3	3.00	22.2	0.68
'Bobinka'	28.3	18.6	3.3	8.0	3.68	9.1	0.33	20.3	2.30	21.0	4.50	35.1	3.80	11.1	1.12
'Vrtka'	81.3	7.9	9.6	32.9	4.42	11.5	0.34	12.3	2.50	21.1	4.60	44.9	2.20	19.2	0.46
'Banana'	38.1	11.2	14.8	5.8	0.94	15.2	0.33	8.3	2.80	15.1	8.70	5.6	2.10	15.1	0.06
'Rošajka'	91.9	11.8	19.4	6.6	2.82	8.5	0.41	15.9	2.80	15.1	8.60	6.0	2.50	21.1	0.15
'Stojankina kletva'	8.4	19.6	6.3	15.3	1.20	18.4	0.23	11.9	2.60	19.9	8.20	5.1	2.10	15.1	0.19
'Lalić'	153.7	16.1	13.4	9.8	5.84	3.7	0.57	4.8	2.80	15.1	4.60	44.9	2.30	21.0	0.44
'Kolonija'	92.6	31.8	16.2	11.4	3.22	14.1	0.47	22.1	1.60	32.3	8.20	5.1	2.10	15.1	0.21
'Sivrija'	109.5	14.1	12.2	3.4	5.00	11.5	0.33	13.6	1.60	32.3	8.10	3.9	2.20	19.2	0.41
'Krivopetlja'	117.2	14.0	8.5	20.9	4.42	14.3	0.58	15.7	2.20	19.2	7.70	6.3	2.60	26.9	0.52
'Strižanka'	145.7	13.3	15.2	8.9	5.53	10.0	0.52	11.0	1.20	35.1	4.50	35.1	2.10	15.1	0.36

For pericarp thickness, four populations had a high diversity index: ‘Čokotanka’, ‘Kurtovska kapija’, ‘Somborka’ and ‘Rošajka’. For the color of the fruit before ripening, ‘Somborka’ and ‘Krivopetlja’ had a medium-high index (0.59). For the shape of the fruit on the longitudinal section, the diversity index was medium to high, while the number of chambers index was high only for ‘Čokotanka’ (0.66) - Table 3.

Table 3. Shannon's index of diversity within the genotype for morphological traits

Genotypes	Weight W/g	Length FL/cm	Width FD/cm	Pericarp thickness/cm PT	Color before ripening CFBM	Shape in longitudinal section SLSF	Number of chambers PNFCH
‘Ruska žuta’	0.82	0.82	0.56	0.51	0.42	0.50	0.38
‘Čokotanka’	0.76	0.65	0.70	0.72	0.38	0.67	0.66
‘Kurtovska kapija’	0.80	0.76	0.65	0.63	0.31	0.33	0.56
‘Žabara’	0.88	0.65	0.74	0.00	0.20	0.61	0.50
‘Somborka’	0.82	0.70	0.74	0.63	0.59	0.50	0.42
‘Bobinka’	0.86	0.58	0.82	0.37	0.31	0.50	0.43
‘Vrtka’	0.86	0.65	0.62	0.54	0.31	0.69	0.42
‘Banana’	0.64	0.82	0.53	0.42	0.20	0.50	0.38
‘Rošajka’	0.80	0.80	0.39	0.63	0.43	0.50	0.42
‘Stojankina kletva’	0.59	0.65	0.53	0.40	0.20	0.67	0.31
‘Lalić’	0.72	0.82	0.41	0.42	0.38	0.50	0.42
‘Kolonija’	0.98	0.70	0.62	0.37	0.20	0.69	0.31
‘Sivrija’	0.76	0.27	0.51	0.51	0.31	0.67	0.20
‘Krivopetlja’	0.74	0.68	0.78	0.63	0.59	0.50	0.38
‘Strižanka’	0.82	0.72	0.82	0.63	0.20	0.50	0.43

The ‘Čokotanka’ population would be suitable for individual selection and reduction of divergence within the population because it had 6 examined traits that had a high value of the diversity index. Populations of ‘S. kletva’, ‘Lalić’ and ‘Strižanka’ should be used for crossing, i.e. recombination of genes to obtain improved properties according to individual selection programs of this species.

Implementation of new technologies that are becoming a basic tool in pepper breeding, combined with traditional selection techniques, among which the diversity index (Shannon-Weaver diversity index H′) is a useful tool for breeders (Kuhn *et al.,* 2016). Shimeles *et al.* (2016), published research on 49 hot pepper genotypes (Ethiopian collections) where he determined a high diversity index for green fruit color and confirmed the average index 0.84. For this property, in our tests, a mostly low or medium high Shannon-Weaver diversity index was determined. For a large number of traits, the obtained results are in agreement with Nsabiyera *et al.,* 2013, who determined, and H′=0.89 for fruit length. In this research, a high index was found for most of the investigated populations.

In order to use the collected domestic pepper populations for selection purposes, grouping was performed based on PCA and cluster analysis, which defined the selection of genotypes that will be candidates for recombination of genetic material. On the basis of Figure 1, B, it was established that the variance of the examined morphological traits is defined by the first two main components with certainty 70.3% (PC1+PC2). Based on the PCA analysis, it was determined that certain genotypes are grouped around a certain trait, but also that certain traits are more or less correlated. According to the results, mass, fruit width and pericarp thickness have the greatest correlation, around which ‘Kurtovska kapija’, ‘Krivopetlja’, ‘Lalić’, ‘Čokotanka’, ‘Somborka’ and ‘Strižanka’ were grouped. Even the length of the vector for individual traits indicated the strength of the influence on variance. The smallest influence on the total variation had the trait color of the fruit before ripening, around which two autochthonous varieties ('Zabara' and 'Vrtka') were grouped. The most divergent genotypes were 'Banana', 'S. kletva', 'Bobinka' and 'Ruska žuta' which took the furthest positions in relation to the average values of the examined population (coordinate start) - Figure 1, B.

It is recommended that genetic divergence be calculated based on principal component scores to avoid discarding some important variables when determining divergence (Do Rego *et al.,* 2003). If the values of the variables are independent, the method based on the Euclidean distance is appropriate, which was applicable in this study and indicated groups of genotypes that were characterized as more or less divergent (Figure 1, A).

Special grouping according to the Euclidean distance between genotypes included the analysis of all genotypes and all tested traits. The examined genotypes were divided into two clusters, one of which has only three genotypes ('S. kletva', 'Banana' and 'Bobinka'), while the other cluster has two subclusters, where one subcluster represents only one genotype ('Ruska žuta'). Within the second subcluster, all other genotypes were classified and within this group, the selection of future parental combinations intended for trait recombination can be planned (Figure 1, A).

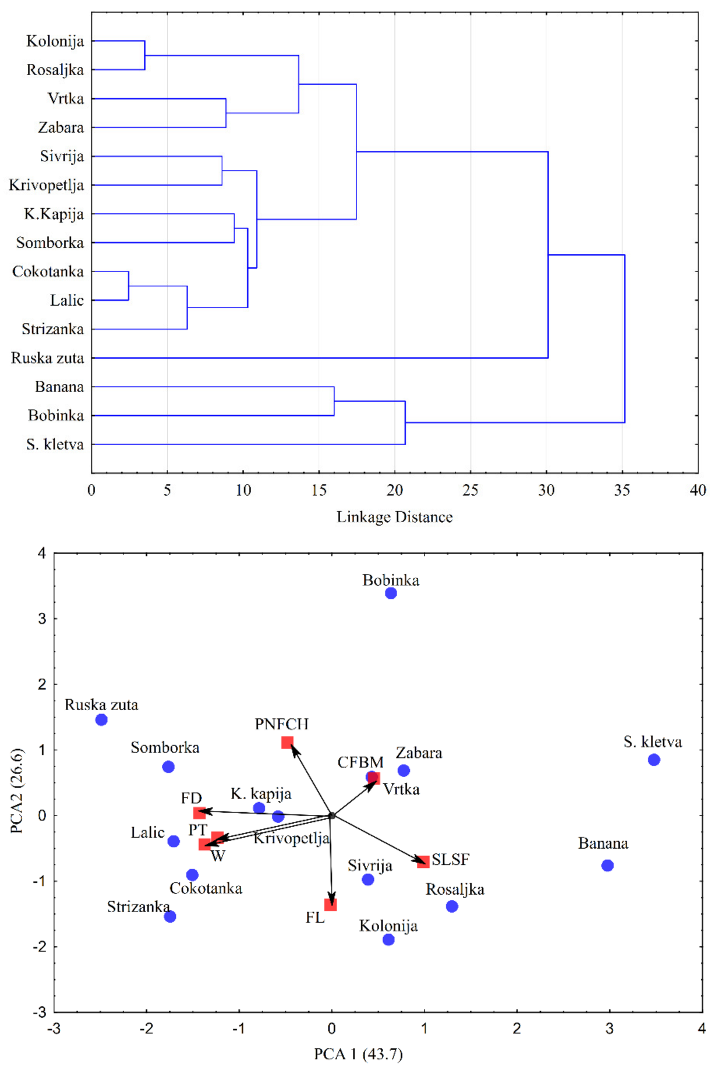

Figure 1. Cluster and PCA analysis of the diversity of fruit morphological parameters of autochthonous pepper populations

The results of research are in agreement with the results of other studies that have systematized autochthonous pepper populations. A high genetic distance and a high positive correlation between morphological parameters were determined for the improvement program of pepper varieties for yield and fruit quality, on 39 genotypes originating from Ethiopia and 20 morphological traits (Geleta *et al.,* 2005). Bozokalfa *et al.* (2009) confirmed that PCA analysis is suitable for grouping genotypes according to different traits in *Capsicum annuum* L., when grouping 48 genotypes of the entire Turkish pepper collection into 7 groups. Zou *et al.* (2004) has grouped 36 local populations on 15 traits in order to do the taxonomic characterisation. In their study Portis *et al.* (2004) have classified 19 ecotypes originating from Italy gene fond, which were classified into 9 clusters using quantitative multivariate techniques. According to Lahbib (2012) in Tunisian local varieties (11) depending on 7 yield traits and its components, PCA analysis determined 3 main components of variability, which was 87% of the total variance. Principal components analysis showed that the first three principal components determined 94.46% of the total variance. Phenotypic variability can be used in a selection program within a population, Barroso *et al.* (2012). In a study by Mirac *et al.* (2022), PCA analysis showed 10 principal components explaining 86% of the total variation. Variations between genotypes mainly happened due to fruit characteristics such as fruit shape and size, which were influenced by environmental factors.

Table 4. Average values of phytochemical parameters of pepper fruit

Genotypes	Carbo-hydrates g/100 g	Essential oils, g/100 g	Ash, g/100 g	Cellulose, g/100 g	β carotene, mg/100 g	K, mg/100 g	Fe, mg/100 g	Vitamin C, mg/100 cm^3	Total phenols (mg GAE/g)	Antioxidant activity IC50 (μg/mL)
'Kolonija'	3.05	0.10	3.55	0.90	0.087	175.00	0.39	212.06	35.43±0.75	13.40±0.55
'Sivrija'	4.22	0.18	4.23	0.74	0.091	184.05	0.55	243.55	31.38±0.15	12.32±0.65
'Kurtovska kapija'	3.78	0.21	3.91	0.81	0.075	192.65	0.45	224.43	29.75±0.25	11.45±0.25
'Vrtka'	4.05	0.25	5.27	0.95	0.099	222.05	0.73	222.37	31.66±0.15	12.65±0.15
'Čokotanka'	3.42	0.15	4.94	0.79	0.088	185.00	0.65	235.25	29.78±0.25	11.55±0.25
'Banana'	3.22	0.18	4.25	0.82	0.100	173.00	0.47	275.05	25.05±0.25	10.85±0.25
'Ruska žuta'	4.05	0.14	3.95	0.92	0.072	167.05	0.43	208.25	24.78±0.05	10.55±0.05
'Krivopetlja'	3.45	0.22	3.55	0.88	0.099	192.35	0.35	234.05	29.05±0.05	11.07±0.35
'Stojankina kletva'	3.78	0.21	3.91	0.81	0.075	192.65	0.45	224.43	29.75±0.25	11.45±0.25
'Rošajka'	3.25	0.32	4.22	0.75	0.120	292.05	0.65	197.05	27.05±0.25	12.05±0.05
'Žabara'	3.43	0.27	3.95	0.85	0.095	197.05	0.53	228.45	30.05±0.05	11.85±0.15
'Lalić'	4.35	0.19	4.77	0.92	0.089	224.15	0.69	220.25	30.25±0.15	12.05±0.15
'Strižanka'	3.35	0.18	3.35	0.55	0.081	198.05	0.55	229.15	30.05±0.05	11.15±0.25
'Somborka'	4.25	0.21	4.23	0.76	0.100	202.25	0.63	221.05	31.02±0.15	12.71±0.15
'Bobinka'	3.05	0.22	3.55	0.65	0.092	165.35	0.57	220.45	30.45±0.25	11.28±0.05

Phytochemical parameters of autochthonous pepper populations give the fruits specificity, according to which their purpose in nutrition is determined. Based on the average values of the parameters, PCA and Cluster analysis of autochthonous pepper populations originating from Republic of Serbia was performed based on fruit quality parameters. The average values of phytochemical substances are shown in Table 4. Carbohydrates ranged from 3.05 g ('Bobinka') to 4.35 g ('Lalić'), essential oils from 0.1 g ('Rošajka') to 0.32 g ('Kolonija'), cellulose 0.55 g ('Strižanka') to 0.95 g ('Vrtka'), ash 3.35 g ('Strižanka') 5.27 g ('Vrtka'), β carotene from 0.072 g ('Ruska žuta') to 0.120 mg/100 g ('Rošajka', respectively), potassium content (K) from 165.35 mg/100 g

('Bobinka') to 292.05 mg/100 g ('Rošajka'), iron content (Fe) from 0.35 mg/100 g ("Krivopetlja') to 0.73 mg/100 g, ('Banana', respectively), total phenols from 24.78±0.05 mgGAE/g ('Ruska žuta') to 35.43±0.75 mgGAE/g ('Kolonija') associated with antioxidant activity from 10.55+0.05 IC50 (µg/mL) ('Ruska žuta') to 13.40+0.55 IC50 (µg/mL) ('Kolonija').

There were two clusters, one of which is represented by 'Rošajka'. The second cluster is divided into two subclusters, one of which is represented by 'Banana'. The second subcluster included all other investigated genotypes and determined two clusters of order II, between which selection can be made for the recombination of fruit quality properties. Lower cluster levels indicate lower divergence values, and according to the Euclidean distance value (Figure 2, A).

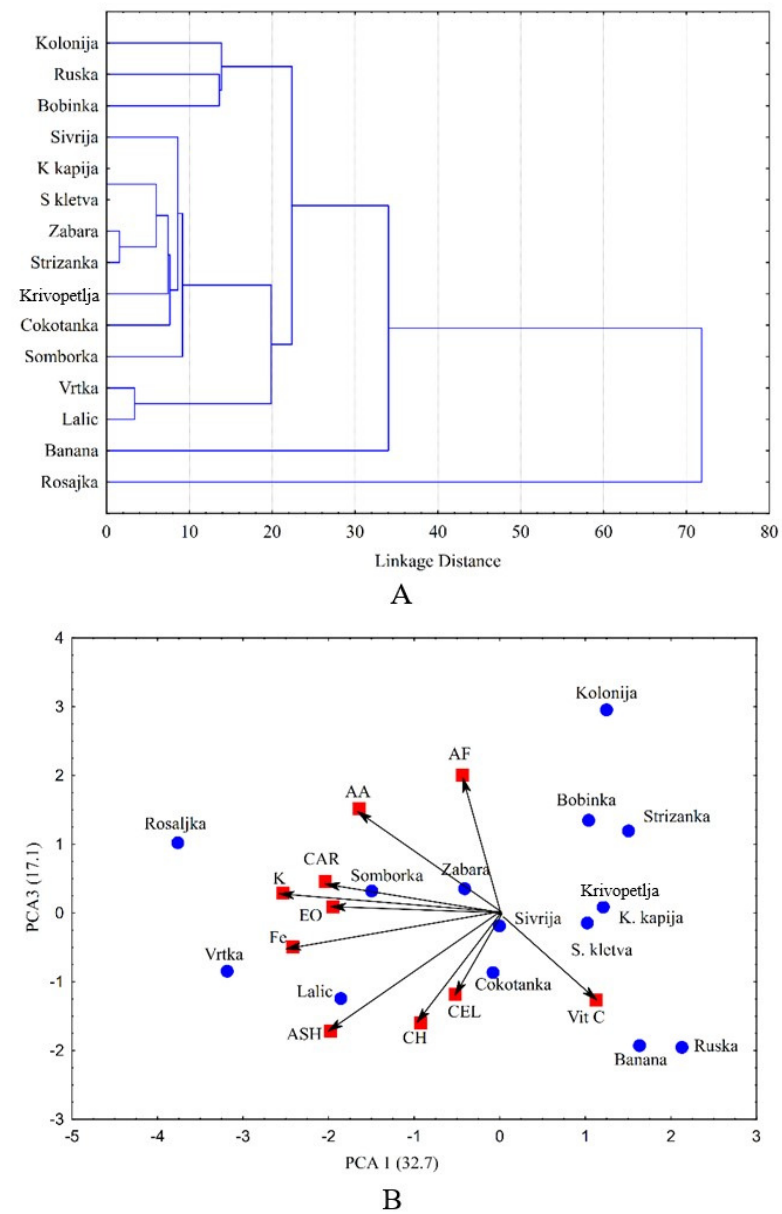

Figure 2. Cluster and PCA analysis of fruit phytochemical parameters of autochthonous pepper populations

Principal component analysis indicates that the first two components determine 49.80% (PC1+PC2) of the total variability of the sample. The arrangement of the vectors that determine the tested properties in the graph indicate the correlation of the properties of β-carotene content, potassium content and essential oil content, while the vitamin C content does not correlate with any of the tested properties. According to the content of all tested traits, the genotype 'Sivrija' was the closest to the average values for all traits, while the variety 'Kolonija' was the furthest from the average values for the tested traits (Figure 1, B).

The two examined autochthonous varieties 'Žabara' and 'Vrtka' belong to 'Vezenka' type of pepper used for consumption in dried form, which is traditional for Serbian and Balkan cuisine, rich in minerals, especially iron ('Vrtka') and antioxidant activity ('Žabara') and represent a genetic resource, which is in agreement with Sandeva, *et al.* (2021) who showed that the collection of 'Vezenka' peppers (dried peppers) is a valuable reservoir of diversity that should be preserved, protected and further exploited in breeding programs. High diversity for the content of vitamin C and other nutritional contents can be considered that these pepper populations are suitable for cultivation due to their high nutritional qualities, which is in agreement with similar studies by Korkutata and Kavaz (2015). Accumulation of carotene in pepper fruits determines the different color in the stage of physiological maturity of the fruit. The color is genetically determined, and it is considered that the lack of the gene for capsanthin (the gene for the formation of the red color of ripe fruits) is a prerequisite for the formation of yellow-fruited peppers (Ha *et al.,* 2007). The content of β-carotene in pepper fruits represents a large source of variation (Wall *et al.,* 2001). The content of β-carotene and other nutritional parameters are largely defined by the time of harvest (fruit consumption at physiological maturity or technologically green fruits). The difference in nutritional values also depends on the content of spiciness, in this research sweet and spicy genotypes were not separated, although in some studies it is emphasized that extracts from sweet varieties have higher average contents of antioxidant activities and concentration of phenolic compounds, than in hot ones (Perucka and Materska, 2007).

PCA and cluster analysis revealed high variability within the analysed germplasm. Fruit traits that had the greatest influence on genotype variability were successfully identified, and four different groups of local varieties were discovered, provided that genotypes that individually classify the cluster branch are excluded, and are related to fruit morphological traits (Figure 1, A), i.e. to three genotype groups if nutritional traits were analysed (Figure 2, B).

Traditional selection techniques in the pepper breeding process, combined with the use of new technologies (Kuhn *et al.,* 2016), become a basic tool in selection for fruit traits. Crossing populations with a higher average trait value and high variance in the F2 generation and rejecting those with different combinations of average values and variance is a common procedure carried out in pepper breeding (Anilkumar *et al.,* 2020).

Conclusions

PCA and cluster analysis revealed high variability within the analysed germplasm. Fruit characteristics that had the greatest influence on the variability of genotypes were successfully identified. The 'Čokotanka' population would be suitable for individual selection and reduction of divergence within the population since its 6 investigated morphological traits had high diversity index, and for selection programs oriented towards the creation of pure lines with improved fruit characteristics. On the other hand, the population of 'S. kletva', 'Lalić' and 'Strižanka' were suitable for obtaining recombination of genes for obtaining improved fruit properties.

Authors' Contributions

Conceptualization (NP and MG); Investigation (MM); Methodology (JM); Software (DT and VGZ); Writing - original draft (JM); Writing - review and editing (JZ). All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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