Original scientific paper

PHENOTYPIC DIVERSITY OF LOCAL PEPPER (*Capsicum annuum* L.) VARIETIES INTENDED FOR DEHYDRATION

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ABSTRACT

Peppers are one of the most divergent group of vegetables globally. In part of the Balkan region, but mostly in Macedonia there are distinct local varieties, intended for drying as a whole fruit on strings and are used in traditional dishes, processed foods and as condiments. A collection of 21 local pepper (*Capsicum annuum* L.) varieties intended for drying in traditional manner, was evaluated for 26 plant traits. The Principal Component Analysis yielded 5 principal components with eigenvalue > 1, explaining 81.48 % of the variability. The first two principal components were highly corelated with 8 plant traits (FL – fruit length, FWg – fruit weight (g), PL – pedicel length (cm), PT – pericarp thickness (mm), PW – plant width (cm), LL – leaf length (cm), DFL-days to flowering, DFR – days to fruiting). The cluster analysis, based on those traits was performed and resulted in grouping the accession into three clusters. The first included accessions with highest values for the analysed traits, while the third cluster included accessions with lowest values for days to fruiting and maturity. The initial characterization of the investigated pepper germplasm, based on qualitative and quantitative plant traits, revealed high variability among the accessions.

Key words: Capsicum, local varieties, diversity, principal component analysis, cluster analysis.

INTRODUCTION

Peppers are one of the most divergent group of vegetables globally. The genus is comprised of 43 species (Barboza et al., 2022), while only 5 are domesticated and dispersed to various extent in different parts of the world, leading with *Capsicum annuum* L. The initial use of this crop was as a spice (Kraft et al., 2014; Perry et al., 2007), while with the domestication and diversification, many forms were developed for various uses (fresh, processed, medicinal, decorative). The Balkan region exhibits a distinctive agricultural tradition for growing pepper varieties that aren't typically identified in other parts of the world (Nankar et al., 2020). Such an example, for part of the region, but mostly for Macedonia, are local embroidered varieties intended for drying as a whole fruit on strings and specific uses in traditional dishes, processed foods and as condiments (Jankulovska et al., 2019). Other local varieties with smooth and semi-wrinkled skin are grown for the same purpose. However, limited research and data are available on the diversity of these local varieties.

The local landraces are specific and their development is a result of long-term production, therefore they become part of the people's tradition (Ficiciyan et al., 2018; Ivanovska et al., 2021; Jankulovska et al., 2019). Furthermore, because of their sensory

attributes like specific aromas and taste, they are maintained by the farmers and are recognized and valued by the local communities (Dwivedi et al., 2019). The initial step in effective conservation and utilization of the potential of the local landraces, is the evaluation of their phenotypic characteristics. This type of data is valuable as for the researchers, as well as for the breeders when choosing material for further selection (de Almeida et al., 2023; Díez et al., 2018). The traditional approaches such as use of descriptor lists for quantitative and qualitative traits in evaluation of the genetic diversity are enhanced with multivariate data analysis and offer reliable data interpretation (Sampaio et al., 2023). The utilization of PCA (Principal Component Analysis) is a common method for determination of the relationship between traits and the variation between genotypes (Bhandari et al., 2017; Bhardwaj et al., 2022). Another useful tool for classification of investigated genotypes based on their similarity is Cluster analysis, that is widely used in assessment of PGR collections (Drvoshanova et al., 2021; Gurung et al., 2020; Martínez-Ispizua et al., 2022; Sandeva Atanasova et al., 2021).

In view of all stated above, the research aim was to assess the phenotypic divergence of 21 local pepper varieties from North Macedonia based on 26 plant traits.

MATERIAL AND METHODS

Plant material was obtained from the collection of the Faculty of Agricultural Sciences and Food in Skopje. A total of 21 local pepper (*Capsicum annuum* L.) varieties were selected based on their local names, indicating their use for traditional method of drying on strings (nizarski, narezen, vezen etc) (Table 1). The study was caried out in Skopje, village Dolno Lisice in randomized block design in three replications with 20 plants per replicate. Planting was done with distance of 30 cm in row and 50 cm between rows. Data was collected in two consecutive years 2017 and 2018. Morphological characterization was made according to the Descriptor for Capsicum (IPGRI, AVRDC, CATIE, 1995) for 13 quantitative and 13 qualitative plant traits.

Table 1. List of investigated Capsicum landraces from North Macedonia (code, local name, origin)

No	Genotype code	Local name	Collection site
1.	FZNH 156	Vezen blag	Erdzelija
2.	FZNH 234	Vezen blag	Konche
3.	FZNH 565	Vezen blag	Mlado Nagorichane
4.	FZNH 129	Vezen blag	Milino
5.	FZNH 510	Luta vezena	Valandovo
6.	FZNH 043	Stara reshka	Neokazi
7.	FZNH 702	Crvena za nizenje	Kalaslari
8.	FZNH 535	Palancanski za nizenje	Ilinden
9.	FZNH 278	Crn za nizenje bugarski	Zhiganci
10.	FZNH 505	Vezen lut	Jurumleri
11.	FZNH 568	Vezen lut	Mlado Nagoricane
12.	FZNH 452	Vezen lut	Kostin Dol
13.	FZNH 151	Nizarski blag	Lozovo
14.	FZNH 014	Piper nizalka	Moroishta
15.	FZNH 023	Nizarski piper	Bigor Dolenci
16.	FZNH 496	Kumanovski vezen	Dolno Lisiche
17.	FZNH 455	Blag vezen	Lukovica
18.	FZNH 339	Dolg crn debel	Teranci
19.	FZNH 427	Luta bukovka	Injevo
20	FZNH 024	Rebrajlija	Vrutok
21	FZNH 532	Vezen lut	Ilinden

Principal component analysis (PCA) was performed on standardized values (mean=0, SD=1) for all quantitative traits for each genotype and the principal components were extracted until eigenvalue was ≥ 1. Cluster analysis was also performed based on 8 quantitative traits, as variables with highest correlation with the first two principal components. Frequency distribution of the qualitative traits was calculated based on 10 observations per genotype and presented for each cluster separately, as well as for the entire collection. The data was statistically analysed with XLSTAT Package 2014.5.03 (Addinsoft, 2014).

RESULTS AND DISSCUSION

Morphological characterization of accessions is first and basic step in crop diversity assessment, particularly for conserved germplasm. Moreover, this type of screening can enable identification of perspective genotypes for further utilization.

The data for the quantitative plant characteristics was subjected to Principal Component Analysis. It was based on 13 quantitative plant characteristics: DFL-days to flowering, DFR – days to fruiting, PH – plant height (cm), PW – plant width (cm), LL – leaf length (cm), LW – leaf width (cm), CL – corolla length (mm), FL – fruit length (cm), FW – fruit width (cm), Index – fruit index, FWg – fruit weight (g), PL – pedicel length (cm) and PT – pericarp thickness (mm), in order to determine which one of them and to what extend are influencing the diversity of the investigated germplasm.

Table 2. Principal component analysis for quantitative traits of the studied local varieties

Traits	PC1	PC2	PC3	PC4	PC5
FL (cm)	0.23	**0.77**	-0.50	0.12	-0.18
FW (cm)	0.03	0.57	0.18	-0.64	-0.08
FWg (g)	**0.71**	0.63	-0.02	-0.21	-0.08
PL (cm)	**0.80**	0.26	-0.14	0.06	0.06
PT (mm)	**0.84**	0.23	0.30	0.00	-0.16
NL	0.31	-0.05	0.63	0.25	-0.25
Cl (mm)	0.40	-0.48	-0.08	-0.10	-0.55
PH (cm)	0.26	0.19	-0.22	0.79	0.19
PW (cm)	**0.76**	-0.14	0.24	0.33	-0.08
LL (cm)	**0.68**	-0.51	-0.33	-0.10	-0.17
LW (cm)	0.38	-0.51	-0.59	-0.21	-0.07
DFL	**0.64**	-0.05	-0.21	-0.21	0.57
DFR	**0.63**	-0.34	0.42	-0.20	0.44
Eigenvalue	4.22	2.35	1.57	1.41	1.03
Variability (%)	32.43	18.10	12.11	10.88	7.96
Cumulative %	32.43	50.54	62.65	73.53	81.48

The first 5 principal components with eigen value > 1, explained 81.48 % of the variation. The first principal component (PC1) contributed with 32,43 % and had highest positive correlations with pedicel length, fruit weight, plant width, fruit index, leaf length, days to flowering and days to fruiting (Table 2). The second principal component (PC2) contributed with 18,10 % and was positively corelated with fruit length. Our results are in agreement with the results obtained by de Almeida et al. (2023) in assessment of Brazilian pepper collection. In their study they evaluated 21 *Capsicum* accessions and the two first PCs explained 36 % and 24 % of the variance, accordingly. Similar results were reported by Bedjaoui et al. (2022) for a collection of 21 hot pepper (*Capsicum annuum*) accessions from Algeria with 26,9 % and 19,5 % contribution of the first two PCs.

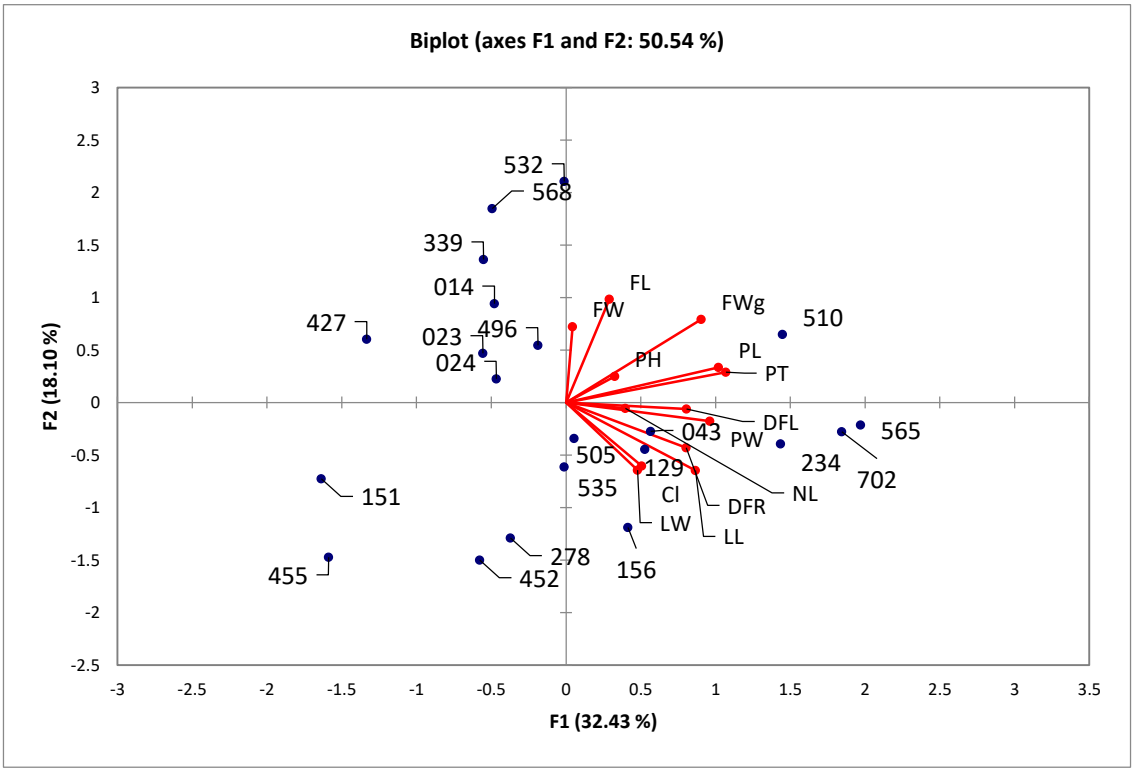

Figure 1. Biplot graphic illustrating relative position of 21 local varieties based on first two principal components

The PCA biplot visualization (Figure 1) of the first two principal components confirms the divergence of the evaluated germplasm, since the accessions are dispersed in all four quadrants. Accessions FZNH455, FZNH452, FZNH151 and FZNH278 are positioned on the lower left side, and had the lowest values for fruit weight pericarp thickness. The accessions positioned on the right side of the plot (FZNH702, FZNH565, FZNH510 and FZNH234) had the highest fruit weight, pericarp thickness, as well as highest values for days to flowering and days to fruiting.

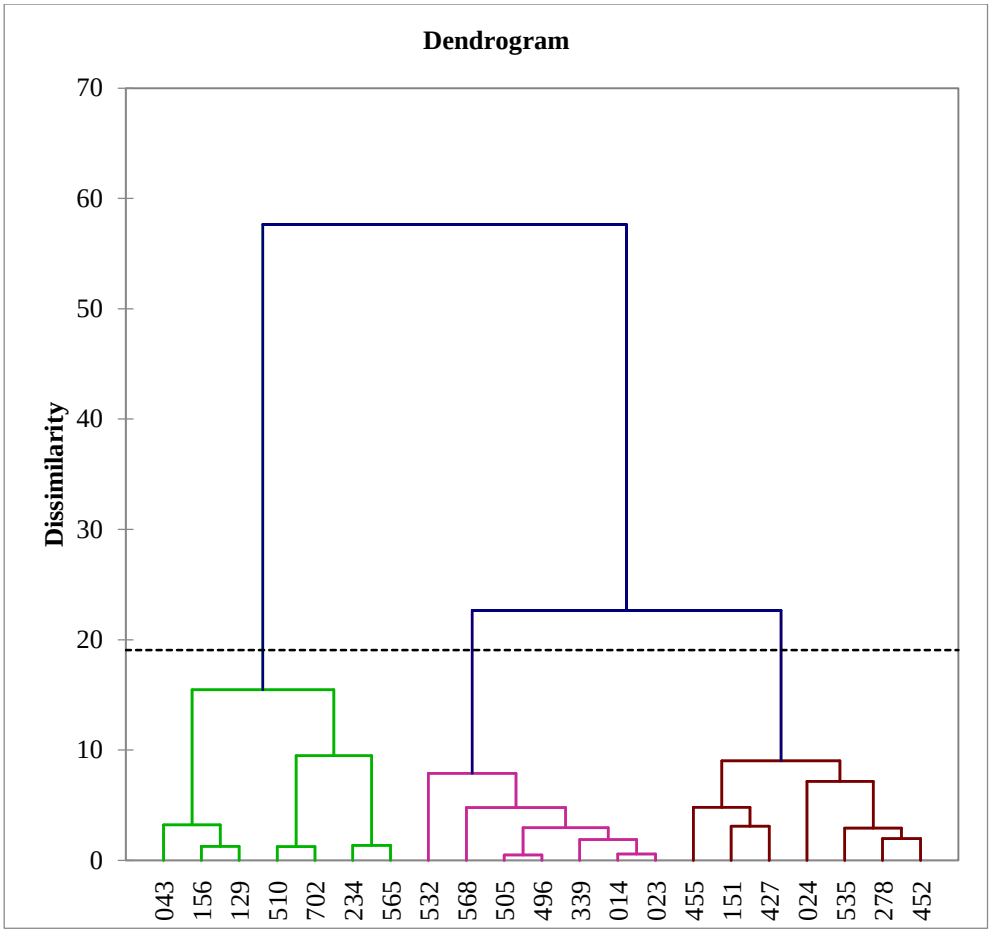

Figure 2. Dendrogram obtained for the analysed fruit traits of 21 analyzed varieties

In order to group the data according to their genetic distance, Cluster analysis was performed based on Euclidian distances and Ward's agglomeration method. Clustering was based on 8 traits that had highest positive correlation (> 0.6) with the first two principal components, as a main source of variability. The material was grouped in three clusters with seven accessions in each cluster (Figure 2).

The first cluster included the accessions with highest values for fruit weight and pericarp thickness (Table 3). In study for inheritance of fruit traits in heirloom cultivars, Vilarinho et al. (2015) found positive correlation between pericarp thickness and fruit mass, therefore this trait is of importance for choosing material for further selection processes. Beside yield, pericarp thickness is linked to fruit quality in the postharvest period making the fruits more resistant to damage caused by transport (Silva & Silva, 2021). The accessions from this cluster can be considered as a good source for selection of traits as good yield components.

Table 3. Average values for the analysed quantitative traits per cluster

Traits	FL (cm)	FWg (g)	PL (cm)	PT (mm)	PW (cm)	LL (cm)	DFL	DFR
Cluster I	15.09	43.09	3.76	3.51	41.33	9.11	82.86	142
Cluster II	16.95	42.24	3.42	3.11	37.01	7.79	78.43	133
Cluster III	13.81	31.10	3.08	2.97	35.83	8.20	77.50	133

Figure 3. Fruit characteristics of investigated accessions from Cluster I

The accessions assigned in the third cluster were characterized with lowest values for the investigated traits except for the leaf length. Considering that the lower number of days to flowering is a desirable trait for earliness, they can be considered as a good source for further selection. The fruit pedicel length is a trait of interest for the analysed germplasm, since the traditional drying method on strings employs needle and plastic thread passing in the base of the pedicel. Since all of the accessions had value above 3 cm for this trait, the value can be considered as acceptable for all clusters.

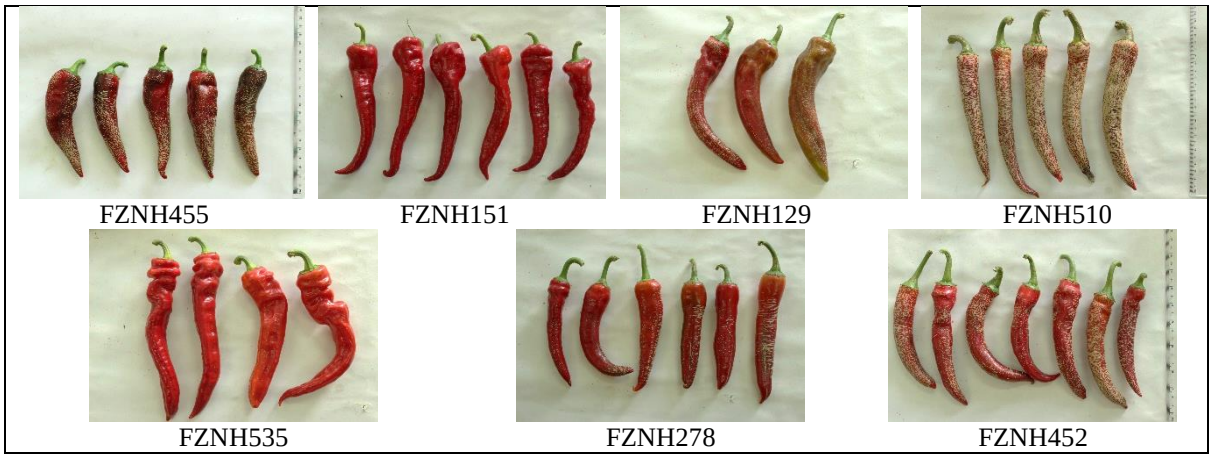

Figure 4. Fruit characteristics of investigated accessions from Cluster III

In terms of qualitative traits (Table 4), most of the accessions had green stem (67 %), followed by green with purple stripes (29 %), while only one accession had purple stems. The stem shape was cylindrical in 62 % and angled in 38 % of the investigated accessions. The presence of nodal anthocyanins was evident in most of the accessions, leading with dark purple (43 %), purple (33 %) and light purple (10 %), while in the remaining 14 % anthocyanins were absent. The presence of purple characteristics determines the presence of anthocyanins, which are classified as nutraceuticals and appetizing agents (Andrade et al., 2020). The leaf colour was predominantly green in 71 % of the accessions, while 19 % had dark green and 10 % had light green leaf colour. Similar results were obtained by Andrade et al. (2020) during characterization of 192 Capsicum accessions from 21 countries. The leaf shape was lanceolate in 57 % and ovate in 43 % of the accessions, while deltoid shape was not observed. The flower characteristics can be very discriminating when evaluating samples from several species of the Capsicum genus, while that is not the case when evaluating samples from a single species.

Table 4. Frequency distribution of qualitative plant traits in the evaluated collection (per cluster and total)

Qualitative traits	***Frequency distribution***			**Total**
	Cluster I	**Cluster II**	**Cluster III**	**%**
Stem color				
Green	5	4	5	67
Green with purple stripes	2	3	1	29
Purple	/	/	1	5
Nodal anthocyanin				
Green	3	0	0	14
Light purple	1	1	0	10
Purple	2	4	1	33
Dark purple	1	2	6	43
Stem shape				
Cylindrical	4	5	4	62
Angled	3	2	3	38
Flattened	/	/	/	/
Leaf color				
Light green	2	/	/	10
Green	5	6	4	71
Dark green	/	1	3	19
Leaf shape				
Deltoid	/	/	/	/
Ovate	3	2	4	43
Lanceolate	4	5	3	57
Flower position				
Pendant	3	1	2	29
Intermediate	4	6	5	71
Erect	/	/	/	/
Stigma exertion				
Inserted	2	4	2	38
Same level	5	3	5	62
Exerted	/	/	/	/
Fruit color at intermediate stage				
White	/	/	/	/
Yellow	/	/	/	/
Green	3	2	2	33
Orange	4	5	5	67
Fruit color at maturity				
Orange	/	/	/	/
Light red	/	/	/	/
Red	6	3	4	62
Dark red	1	4	3	38
Fruit cross-sectional corrugation				
Slightly corrugated	6	4	6	76
Intermediate	1	3	1	24
Corrugated	/	/	/	/
Fruit surface				
Smooth	0	1	1	10
Semi-wrinkled	1	2	4	33
Wrinkled	/	/	/	/
Embroidered	6	4	2	57
Ripe fruit persistence -Pedicel with fruit				
Slight	2	1	/	14
Intermediate	4	4	4	57
Persistent	1	2	3	29
Ripe fruit persistence -Pedicel with stem				
Slight	4	2	2	38
Intermediate	3	5	4	57
Persistent	/	/	1	5

Considering that all evaluated accessions belong to *Capsicum annuum* species, some of the investigated traits are common for all representatives of this specie and they did not affect the divergence of the collection. Therefore, they are not presented in the data analysis. Thus, the number of flowers per axil in the analysed collection was 1, rarely 2, which is a typical characteristic of the *annuum* species. Also, the corolla colour of all investigated accessions was white, with white spots, white filaments and purple anthers. This is in agreement with the description by Barboza et al. (2022) for the *C. annuum* species. Regarding the flower position, most of the accessions (71 %) had intermediate and 21 % had pendant position. The position of the stigma is a trait of significance for assessing risk of cross pollination, that in pepper under various conditions can range between 2 and 90 % (Pickersgill, 1971). In this collection the stigma was on same level with the anthers in 62 % of the accessions, while in 38 % was inserted. Similar results were reported by Ristovska et al. (2013) for evaluation of flower traits in heirloom pepper varieties from south-eastern Balkan region. The fruit colour at intermediate stage was orange in 67 % and green in 33 % of the accessions. At maturity, the accessions had red (62 %) and dark red (38 %) colour of the fruits. Fruit colour at maturity is important trait in estimating time of harvest, particularly for peppers intended for processing and dehydration, since physiologically ripe fruits have best colour retention in the postharvest period (Bridgemohan et al., 2018). All investigated accessions had elongated fruit shape, while the cross-sectional corrugation varied between slightly corrugated (76 %) and intermediate (24 %). The fruit surface was smooth in 2 accessions, semi-wrinkled in 7 accessions, while 12 accessions were recorded as embroidered (with striations on the skin). The persistence of the fruit with the stem is a trait of importance for facilitation of the harvesting processes. In this collection, most of the accessions were recorded as intermediate (57 %), followed by slight (38 %) and only one accession (5 %) was graded as persistent. The persistence of the pedicel with fruit is significant for the postharvest manipulation of the fruits, depending weather the fruit will be dried as a whole (on strings) or they will be cut, powdered etc. For this trait, 57 % of the accession were recorded as intermediate, 29 % as persistent and 14 % as slight.

CONCLUSIONS

The initial characterization of the investigated pepper germplasm, based on qualitative and quantitative plant traits, revealed high variability among the accessions. Since these local varieties have not been previously studied, this research will provide a base for development of effective conservation and selection strategies. The accessions from the first cluster can be of interest for further selection for yield components, while the accessions from the third can be source of genes for earliness. However, broadening the knowledge for the biochemical composition of the fruits is recommended as a following step for successful utilization of the conserved germplasm.

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