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# DIVERSITY AND RELATIONSHIPS IN KEY TRAITS IN PEPPER COLLECTION OF NATIONAL GENEBANK OF BULGARIA

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***Abstract***

*To investigate the structure of genetic diversity in pepper germplasm resources in Bulgaria, we compared 52 accessions from GenBank (local varieties and local cultivars). The accessions were characterized based on 14 morphological and vegetative characteristics and ascorbic acid content, fruit dry matter content and sugars. The results of the present study revealed a relatively high level of diversity in the collection. The most significant correlations were found between fruit weight and fruit dimensions - length and width. A positive correlation (>0.5) was reported between vitamin C, dry weight and sugars. A hierarchical cluster analysis was performed and the genotypes were grouped into three clusters. Biplot analysis showed that the genotypes B4E0032, SR and BR from cluster 3 showed a preference for larger values of fresh fruit weight. The significant morphological and biochemical differences between the evaluated specimens are the basis for further breeding programs and a major source of agricultural biodiversity. The evaluation of pepper varieties in the Bulgarian Gene Bank is necessary and important in supporting the development of Capsicum breeding strategies*

***Key words**: Capsicum annuum, morphology, diversity, characterization.*

## INTRODUCTION

Conservation and sustainable use of pepper genetic resources are key to responding to climate change and increasing global food demand in the coming decades. Conservation of genetic diversity is an essential prerequisite for improving plant breeding programs and developing new varieties with desirable agronomic traits and for expanding the genetic base of this economically important crop (Christov, N. et al., 2021). The genetic diversity of germplasm from local varieties is considered one of the criteria for selection of parents for breeding new pepper varieties, due to their competitive advantage (Lanteri and Barcaccia, 2006). Local varieties have higher genetic variability between different germplasm groups, as well as better adaptation to the environment and are an indispensable source of highly co-adapted genotypes. They represent a dynamic and essential component of overall agricultural biodiversity, valued as a source of traits that can be used in scientific breeding programs and to improve the productivity of new crop varieties. Cultivated Capsicum species provide a great diversity in morphological, biological and consumer characteristics of pepper, especially in Central and South America, their geographical origin. As a result of evolution, domestication, and artificial and natural selection in primary and secondary centers of diversity (Garcia B. et al., 2015; Tripodi et al., 2021). Indeed, more than five centuries of pepper breeding in Europe have resulted in a wide variety of local varieties adapted to a wide range of agroclimatic conditions (Garcia et al., 2015).

The Balkans, including Bulgaria, are considered a secondary center of pepper diversity, especially for *C. annuum,* where local forms (local varieties) with diverse phenotypes and qualities have been formed due to specific agroclimatic conditions and growing traditions (Krasteva, L. et al., 2012; Tsonev, S. et al., 2017; Christov, N. et al., 2021). Locally maintained and well-adapted local pepper varieties can still be found in small farms and village yards in Bulgaria and other Balkan countries (Krasteva L. et al., 2009). Characterization of the agro-biodiversity of pepper that exists in Balkan germplasm collections can assist breeding programs by providing more insight into fruit quality. Comprehensive germplasm characterization provides insight into the selection of promising breeding lines that could improve fruit quality and nutritional value of future pepper cultivars. The aim of the study is to assess the genetic diversity of local genotypes of sweet and hot peppers from the Bulgarian Genebank in terms of morphological, biological chemical characteristics as a basis for future breeding programs and a source of agricultural biodiversity.

## MATERIALS AND METHODS

The experimental material included samples of 52 local pepper varieties cultivated in Bulgaria (Table 1), both hot and sweet types.

All experiments were conducted on sandy-clay soil at the Institute of Plant Genetic Resources, Sadovo, Bulgaria.

The experimental design was a randomized complete block with three replications; each plot consisted of 20 plants. Data were collected from 10 plants. Five unharvested plants were left to collect data on full fruit maturity, and five were left to minimize edge effects. Soil preparation, fertilization, and plant protection were carried out according to standard practices for pepper cultivation in Bulgaria.

The data on morphological and physiological traits were determined according to the descriptors of the International Plant Genetic Resources Institute for Capsicum (IPGRI, 1996). The statistical analysis of the data was performed using IBM SPSS Statistics 19.

## RESULTS AND DISCUSSIONS

In this study, 52 pepper genotypes from the National Gene Bank of Bulgaria were characterized. As with many plant species, the agronomic and morphological characteristics of pepper genotypes originating from various sources have been studied by numerous researchers (Mutlu and Agan E., 2015; Bozokalfa and Eşiyok, 2010; Cankaya, S. et al., 2010; Virga et al., 2020; Başak, 2019; Taş and Balkaya, 2021; Christov, N. K. et al., 2021; Nankar A.N. et al., 2020).

### *Descriptive statistics*

The mean, maximum, and minimum values for 10 agromorphological traits revealed a wide range of phenotypic variation among the evaluated samples (Table 1).

The analysis results showed that plant height ranged from 45 cm to approximately 60 cm, with most of the samples falling within the 50-58 cm range. The number of branches varied between 3 and 5.

Table 1. Descriptive statistics
of the agromor- phological traits

| **Trait** | **Min.** | **Max.** | **Mean** ± SEM | **S.D.** |
|---|---|---|---|---|
| Plant Height | 32.40 | 69.0 | 55.73±0,63 | 0.94 |
| Embranchment | 2.00 | 6.60 | 3.64±0,08 | 0.14 |
| Leave Length | 6.78 | 17.74 | 11.62±0,22 | 0.36 |
| Leave Width (cm) | 3.20 | 9.42 | 6.26±0,13 | 0.21 |
| Fruit Length (cm) | 3.68 | 19.54 | 10.60±0,20 | 0.66 |
| Fruit Width (cm) | 1.60 | 8.20 | 4.54±0,19 | 0.29 |
| Wall Thickness ( | 0.10 | 0.78 | 0.36±0,01 | 0.02 |
| Locules | 2.00 | 5.00 | 2.68±0,07 | 0.09 |
| Fruit Weight (g) | 7.00 | 123.40 | 56.67±2,20 | 4.98 |
| Flowering Earliness | 62.00 | 89.00 | 73.08±1,04 | 1.14 |
| Maturity Earliness | 44.00 | 64.00 | 49.23±0,80 | 0.65 |
| Vitamin (%) | 13.62 | 332.98 | 193.55±2,82 | 7.99 |
| Total Sugars (%) | 2.39 | 7.82 | 4.86±0,05 | 0.20 |
| Fruit Dry Matter (%) | 6.61 | 18.00 | 10.35±0,60 | 0.39 |

Fewer branches indicate a more compact shrub form. The leaf size trait showed significant variation. Specifically, leaf length ranged from 7 to 11.5 cm, while leaf width ranged from 3.3 to 6.5 cm. A larger leaf area suggests more active photosynthetic activity and better overall development. The greatest variation was recorded for fruit weight and vitamin C content The measured traits showed high variability, confirming previous studies (Bozokalfa and Eşiyok, 2010; Başak, 2019).

### *Correlation analysis*

Correlation relationships between the variables are presented in Figure 1.

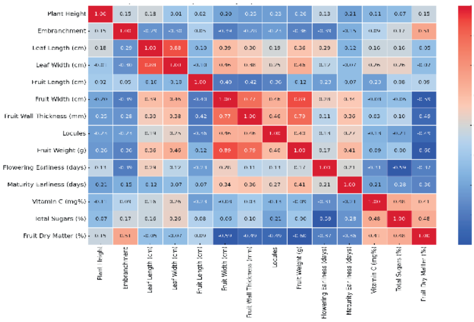

Figure 1. Correlation matrix

Strong positive correlation (~0.7-0.9) was observed between fruit weight and fruit size - specifically, length and width. Our results are consistent with those reported by Matthew et al. (2004) and Ullah et al. (2011) who also found positive correlations for these traits. Positive correlation (>0.5) was also recorded between vitamin C content, dry matter, and sugar content.

Similar results were reported by Naskar et al. (2020).

Moderately positive correlation was found between fruit weight and fruit wall thickness, and a weak positive correlation between number of branches and vitamin C content, as well as between fruit weight and number of locules.

An inverse correlation was observed between earliness and vitamin C content. A negative correlation was also reported by Naskar et al. (2020)

***Principal component analysis***

PCA was used to identify the differences between the samples as a measure of phenotypic and component variability, and to examine the relative contribution of different biomorphological traits to overall diversity. Principal Component Analysis (PCA) was applied to the studied traits.

According to the results of this study, it was established that traits in the first three principal components (Table 2) should be considered, as they explain a significant portion of the total variation and have loading values greater than 0.3 (Brown, 1991).

Table 2. Eigen vector for Agronomic Characters of the first three Principal Components

| | PC1 | | PC2 | | PC3 |
|---|---|---|---|---|---|
| Fruit Width | 0.42 | Total sugars | -0.49 | Leave Length | 0.45 |
| Fruit Weight | 0.40 | Vitamin % | -0.44 | Plant Height | 0.49 |
| Fruit Wall Thickness | 0.38 | Leave Width | -0.42 | Flowering Earliness | 0.42 |
| Fruit dry matter | -0.35 | Leave Length | -0.37 | | |
| Locules | 0.29 | | | | |

The role of biomorphological characteristics in understanding the divergence between pepper populations was clearly demonstrated in our study and has been previously reported in transcontinental studies of *Capsicum*, with observations made in Brazilian *C. chinense* (Bianchi P.A. et al., 2020), nine Bulgarian accessions (Tsonev S. et al., 2017), the Turkish pepper collection (Bozokalfa et al., 2009), Ugandan hot pepper (Nsabiyera V. et al., 2013), and the Serbian pepper collection (Danojevic D. et al., 2017). Based on PCA analysis and the eigenvectors for the first three principal components, we can make the following summary Principal Component 1 (PC1) explains the largest portion of the variation. Key traits: fruit width (0.42), fruit weight (0.40), fruit wall thickness (0.38), fruit dry matter (-0.35), and locules (0.29). PC1 primarily captures differences related to fruit size and structure. Varieties with larger, heavier fruits and thicker walls have high values in this component. Principal Component 2 (PC2) captures the next level of variation. Key traits: total sugars (-0.49), vitamin C percentage (-0.44), leaf width (-0.42), and leaf length (-0.37). PC2 reflects differences related to leaf morphology and sugar/vitamin content. Varieties with higher sugar and vitamin content and broader leaves score higher on this component. Principal Component 3 (PC3) captures more subtle differences. Key traits: leaf length (0.45), plant height (0.49), and flowering earliness (0.42). PC3 distinguishes varieties by plant stature, leaf length, and phenological traits such as early flowering - i.e., traits associated with vegetative growth and development.

Fruit physical characteristics (size, weight, wall thickness) are most significant in PC1 and explain the greatest variation among the varieties. Sugar and vitamin content, as well as leaf morphology, are critical in PC2. Plant habit and phenology (height and flowering) are weaker contributors but still distinguish varieties in PC3. Figure 2 presents a heatmap comparing two different clustering methods used to group the pepper varieties:: PCA clustering, based on Principal Component Analysis and the K-means algorithm.

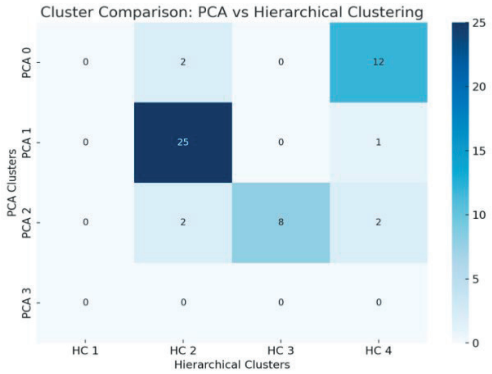

Figure 2. Cluster comparison PCA vc HC

Hierarchical clustering, based on a hierarchical method that creates a dendrogram and stepwise merges the closest groups.

The rows show the groups formed by PCA. The columns show the groups formed by hierarchical analysis. The numbers in the cells indicate the number of varieties assigned to each specific combination of both clustering methods. In our study, most of the varieties (32) appear along the diagonal of the matrix (from the top left to the bottom right), indicating that the two methods yield similar results.

Twelve accessions fall outside the diagonal, meaning they were grouped differently by the two methods.

### *PCA Biplot: PC1 vs PC2*

This biplot combines the information from the PCA analysis: the points represent individual accessions. The red vectors indicate the contribution of each variable to the principal components - the length and direction of each arrow reflect the strength and direction of influence. The biplot analysis of the quantitative traits revealed significant variability among the accessions. The biplot showed that characteristics related to fruit size and structure - such as fruit weight, wall thickness, and fruit width - contributed strongly to PC1while variables such as sugars, vitamin C, and dry matter were more closely associated with PC2. The genotypes PR, BR1, and B0E0341 showed their potential for flowering earliness, maturity earliness, and number of locules.

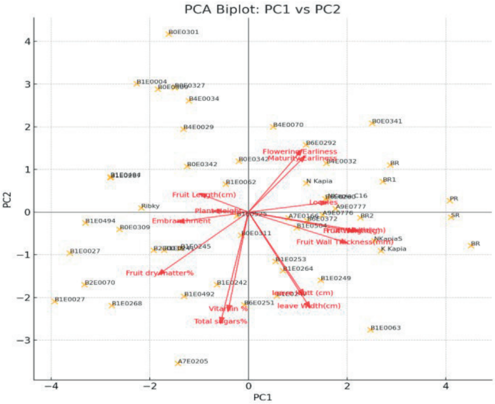

Figure3. PCA Biplot: PC1 vs PC2

SR, BR, and B1E0063 formed a group with larger fruits, thicker walls, and broader and longer leaves.The genotypes were distributed across all four quadrants of the biplot.

Six genotypes - A7E0205, BOE0309, B1E0027, B1E0494, B1E0268, and B2E0070 - formed a separate group on the negative side of PC1 due to their low content of sugars, vitamin C, and dry matter. Four genotypes - BOE0301, B1E0004, B2E0034, and B1E02422 - were grouped on the positive side of PC2 because of their short fruit length.

The remaining 39 genotypes were positioned closer to the center of both axes, as their traits showed similar values.

### *Cluster analysis*

Based on the cluster analysis, the varieties included in this study were grouped into three main clusters (Figure 4), according to the average levels of variation between the accessions generated from the genetic distance matrix.

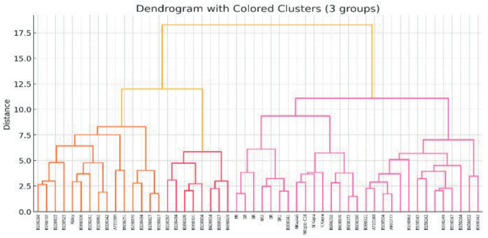

Figure 4. Dendrogram based on 13 quantitative traits of 52 pepper genotypes

Cluster I included 13 accessions, Cluster II had 8, and Cluster III contained the majority-29 accessions. Cluster I: These were medium-height plants with fruits of average weight, but very high vitamin C and sugar content. They have excellent taste - ideal for fresh consumption and as functional foods. They are typically mid-season varieties. Cluster II: These are low to medium-height plants that produce small to medium fruits (longer than they are wide). They have thin walls and low fruit weight. They mature early and are suitable for early production. Cluster III: These are tall plants with larger leaves. The fruits are large, heavy, have thick walls, and contain 3 or more locules. They have high dry matter content. These are usually late varieties, suitable for industrial processing and market-oriented production.

## CONCLUSIONS

The results of the present study revealed a relatively high level of diversity within the collection. This information may be useful for initiating a conservation strategy, as well as for supporting an informed breeding and improvement program for pepper.

The most significant correlations were identified between fruit weight and fruit dimensions - length and width - with a strong positive correlation observed. Positive correlation (>0.5) was also noted between vitamin C, dry matter, and sugar content. Hierarchical cluster analysis was conducted, and the genotypes were grouped into three clusters. Principal Component Analysis (PCA) identified three main components.

The biplot analysis showed that genotypes B4E0032, SR, and BR from Cluster 3 showed a preference for higher values in fresh fruit weight.

The results obtained can support the identification of potential genotypes for further breeding or recommendation for direct use in production. The evaluation of pepper varieties in the Bulgarian Gene Bank is necessary and important for developing effective breeding strategies for *Capsicum*.

## REFERENCES

Başak, H. (2019). Agronomic and morphological characterization of Kırşehir local green long pepper (*Capsicum annuum* L. var. *longum*) Populations. Kahramanmaraş Sütçü İmam *Üniversitesi Journal of Agriculture and Natural Science*, 22(2), 202-216.

Bozokalfa, M.D., Eşiyok, D., (2010). Determination of genetic diversity by agronomic traits in pepper (*Capsicum annuum* L.) accessions. *Journal of Ege University Faculty of Agriculture*, 47(2), 123–134

Bozokalfa MK, Esiyok D,Turhan K. Patternsof (2009). Phenotypic variationin a germplasm collection of pepper (*C. annuum* L.) from Turkey. *Span J. Agric.Res.2009*; 7(1): 83–95.https://doi.org/10.5424/sjar/ 2009071-401

Brown, J.S, (1991). Principal component and cluster analysis of cotton cultivar variability across the U.S. Cotton Belt. *Crop Science*, 31, 915-922.

Cankaya, S., Balkaya, A. and Karaagac, O. (2010) Canonical Correlation Analysis for the Determination of Relationships between Plant Characters and Yield Components in Red Pepper [*Capsicum annuum* L. var. *conoides* (Mill.) Irish] Genotypes. *Spanish Journal of Agricultural Research*, 8, 67–73. http://dx.doi.org/10.5424/sjar/2010081-1144

Christov, N. K., Tsonev,S., TodorovaV.& Todorovska, E.G. (2021). Genetic diversity and population structure analysis – a prerequisite for constructing a mini collection on Balkan *Capsicum annum* germplasm. *Biotechnology & Biotechnological Equipment,* 35(1), 1010-1023 https://doi.org/10.1080/13102818.2021.1946428

Danojevic D, Medic-Pap S., Cervenski J. NS. (2017) Pepper varieties in a mutilvariate fruit analysis. In: Proceed ings of the VIII International Agricultural Symposium, AGROSYM. 2017. pp. 495–500.

Garcia, B T., Coleto Martınez, J. M., and Velazquez Otero, R. (2015). Historias de las Plantas II: la Historia del Pimiento. *La agricultura y la ganaderıa extremeñas,* 241–254.

Krasteva L, Stoilova T, Varbanova K. (2009). Bulgarian Landrace inventory - significance and use. In: Veteläinen M, Negri V, Maxted N, editors. *European landraces: on-farm conservation, management and use*. Rome: *Biodiversity International*; 2009. p. 53–68.

Krasteva L, Pandeva R, Rodeva R (2012). Pepper as a target object of see-era.net project. *Acta Hortic*. 2012; 960_21:151–158

Lanteri, S. and Barcaccia G. (2006). Molecular marker based analysis for crop germplasm preservation. In: Ruane, J.; Sonnino, A. (Ed.). *The role of biotechnology in exploring and protecting agricultural genetic resources*. Rome: FAO, p. 55–66.

Matthew D, Doijode SD, Reddy KM (2004). Correlation and path coefficient analysis in five species of Capsicum. Capsicum and Eggplant *Newsletter* 2004; 23: 57–60.

Mutlu S.S. and Agan E. (2015). Effects of Paclobutrazol and Pinching on Ornamental Pepper *HortTechnology*, vol. 25: (5), 657–664 doi: https://doi.org/10.21273/HORTTECH.25.5.657

Nankar AN, Todorova V., Tringovska I., Pasev G., Radeva-Ivanova, V., Kostova D. (2020). A step towards Balkan *Capsicum annuum* L. core collection: phenotypic and biochemical characterization of 180 accessions for agronomic, fruit quality, and virus resistance traits. Ezura H, editor. PLoS One. 2020;15(8):e0237741

NsabiyeraV, Logose M, Ochwo-Ssemakula M, Sseruwagi P, Gibson P,Ojiewo C. (2013). Mor phologicalchar acterization of local and exotic hot pepper (*C. annum* L.) collections in Uganda. *Bioremediation, Biodi versity and Bioavailability*. 2013; 7(1): 22–32. http://oar.icrisat.org/id/eprint/6366

Taş, K., Balkaya, A. (2021). Determination of morphological variation by principal component analysis and characterization of the *Capsicum chinense* genetic resources. Ekin *Journal of Crop Breeding and Genetics*, 7(2), 86–105.

Tsonev S, Todorova V, Groseva S, Popova T., Todorovska E. (2017). Evaluation of diversity in Bulgarian pepper cultivars by agronomical traits and ISSR markers. *Genetika,* 49(2): 647–662.

Tripodi, P., Rabanus-Wallace, M. T., Barchi, L., Kale, S., Esposito, S., Acquadro, A. (2021). Global range

expansion history of pepper (*Capsicum* spp.) revealed by over 10,000 genebank accessions. *Proc. Natl. Acad. Sci.* U. S. A. 118, 1–9.
doi: 10.1073/ pnas.2104315118

Virga G., Licata M., Benedetto B., Tuttolomondo T., Sabatino L., Leto C., La Bella S. (2020). Agro-Morphological Characterization of Sicilian Chili Pepper Accessions for Ornamental Purposes. *Plants* 9(10), 1400; https://doi.org/10.3390/plants9101400

Ullah MZ, Hasan MJ, Saki AI, Rahman AH Biswas PL. (2011). Association of correlation and cause-effect analysis among morphological traits in chili (*C. frutescence* L.*). Intl. J. BioRes*.; 10(6): 19–24.