

Article

# Assessment of Fruit Quality and Fruit Morphology in Androgenic Pepper Lines (*Capsicum annuum* L.)

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## ABSTRACT

Anther or microspore culture induced haploid and double haploids (DH) are increasingly being utilized by breeders since it can shorten breeding time by achieving complete homozygosity within a single generation. Pepper (*Capsicum annuum* L.) is one of the most important vegetables, distinguished by its high level of heterozygosity, making the breeding process very laborious and long. Fourteen different DH lines were obtained as a result of anther culture of four parental genotypes. Data from different plant and fruit morphological traits as well as quality traits including vitamin C, dry matter content, total polyphenols and antioxidant activity were collected. A total of 47 different descriptors for fruit morphology and color were characterized using Tomato Analyzer v. 3 software. Findings from this research revealed significant variation of fruit morphology, quality and productivity traits between DH lines and their respective parental genotypes. Among these studied 14 DH lines, 42.9% were superior to the parental genotypes for fruit weight, width, fruit wall thickness, and usable part of the fruits. As compared to parental genotypes, DH lines exhibited higher values for tested fruit quality traits and dry matter content. Multivariate analysis allowed us to identify the trait combination that contributed to the total variation. A total of eight principle components (PCs) explained 95.4% variation with PC1 and PC2 contributing 32.4% and 21.1%, respectively. Pepper androgenesis clearly indicates its usefulness as a well established technique that can allow pepper breeders to save the time and breeding resources by expediting the breeding process. Our research findings prove the advantages of pepper androgenesis to utilize the diversity of pepper genetic resources and development of novel pepper breeding lines to utilize in future breeding.

## Open Access

Received: 06 December 2019

Accepted: 22 January 2020

Published: 23 January 2020

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**KEYWORDS:** pepper; anther culture; double haploids; total polyphenols; Tomato Analyzer

## INTRODUCTION

Pepper is an economically important crop from the *Solanaceae* family characterized by wide variation for fruit color, shape and size. In recent years, studies have focused on to fruit quality traits with enhanced antioxidant concentrations and health-promoting properties [1]. Biodiversity using advanced cytological and tissue culture methods has been proven effective to improve pepper germplasm with novel value added traits. Production of haploids and double haploids (DHs) is an important plant-breeding tool that allows rapid recovery of unique homozygous genetic recombinants, and quick detection of recessive mutations. Double haploid lines are immensely valuable to speed up the breeding process. They have been identified as essential breeding material for crop improvement due to its proven practical applications for the development of hybrids that display maximum heterosis and/or improved traits [2,3].

Anther culture is the most applied method used in pepper to obtain haploids and DH plants. Success of this technique is determined by numerous factors such as genotype, physiological status of donor plant, pollen development stage, culture media composition, anther pretreatments and other unknown factors [4–6]. Spontaneous or induced genome doubling of haploid allows development of fully homozygous plants with unique genetic recombinants [7].

The morphological evaluation of DH lines allows the confirmation of genetic homogeneity of a single line; it also makes it possible to present the diversity across different lines obtained in anther culture [8,9]. This variation between DH lines reflects the genetic diversity of microspores, which results from random gene segregation in meiosis, and is one of the most important conditions of in vitro androgenesis practical applicability to plant breeding [10]. On the other hand, studies associated with quality traits including sugar, ascorbic acid or vitamin C content, total polyphenols as well as antioxidant properties of DH lines could also be helpful for selecting the regenerants [11]. Inclusion of this genetic diversity in pepper breeding programs leads to selection of individuals with valuable combination of agronomic and fruit quality traits [12,13]. Hence this study was designed to evaluate fruit quality characters and fruit morphology of 14 diverse androgenic pepper lines.

## MATERIALS AND METHODS

The experimental work was carried out during 2018–2019 at Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria. Fourteen DH lines obtained as a result of self-pollination of anther-derived regenerants and four parental genotypes—one hybrid “202” and three varieties—“Slonovo uvo”, “Stryama” and “Zlaten medal 7”. Three of the evaluated lines originated from hybrid “202”, two lines from variety “Slonovo uvo”, five lines from variety “Stryama” and four lines from variety “Zlaten medal

7". The plants were grown under field conditions with furrow surface irrigation in a 70/15 cm scheme. The DH lines and parental genotypes were evaluated during two consecutive years in two replications. Each replication consisted of 10 plants. The fruits were harvested at maturity. Plant productivity (g), average fruit weight (g), fruit length (cm), fruit width (cm), usable part of the fruit (g) and fruit wall thickness (mm) were determined. These traits were evaluated on five plants per replication and three fruits per plants.

Eight fruits per genotype were prepared for high-throughput phenotyping using Tomato Analyzer (TA) software. Fruits were cleaned, cut into longitudinal and latitudinal sections, scanned at 300 dpi and analyzed using TA for a total of 47 different fruit morphometric [14] and colorimetric traits [15].

A sample of ten randomly selected fruits from each genotype were used for analysis of the following fruit quality traits: dry matter (DM), ascorbic acid or vitamin C (Vit C) content, Total polyphenols (TP) and ferric-reducing antioxidant power (FRAP). Fruits were rinsed three times with distilled water and were wiped. Half of the pericarp was freshly homogenized to juice and used for analysis of dry matter (by oven drying at 105 °C to a constant weight) and vitamin C content (by Tillman's reaction [16]). Half of the pericarp was lyophilized, powdered and used for analyzes of total polyphenols and antioxidant activity. Total polyphenols and ferric-reducing antioxidant power (FRAP) extraction procedures were performed according to the optimized method described by Atanasova et al. [17]. Total polyphenols were quantified according to the Singleton and Rossi [18] method. The FRAP antioxidant activity was measured following the procedure originally described by Benzie and Strain [19].

### Statistical Analysis

The fruit quality data were analyzed using analysis of variance (ANOVA) and post-hoc Duncan test to identify between accession differences. Fruit image data were transformed by log transformation and were statistically analyzed using ggplot2 package of R program. Correlation of fruit morphometric and colorimetric traits was determined by multivariate technique of principal component analysis (PCA). The correlation and PCA were performed based on the basis of the average values of the investigated parameters. The PCA was used to determine between accession variation and various PCA parameters were estimated using ggplot2, missMDA, FactoMineR, and Factoextra R packages.

## RESULTS

The results from manual measurements of fruit morphology and plant productivity showed significant phenotypic homogeneity within androgenic lines and considerable variation for main fruit characters

and productivity among different androgenic lines of same background (Table 1). The lines originating from F<sub>1</sub> “202” were distinguished with higher values of the measured fruit morphological traits in comparison to the parental genotype, while the highest productivity, Vit C, dry matter, and antioxidant activity was observed in line “211”. The highest value of TP was measured in line “212” (Table 2). The statistical analysis of the lines deriving from variety “Slonovo uvo” showed that line “214” exceeded the parental genotype for studied fruit morphology traits (Figure 1A,B). In terms of Vit C content, TP, and antioxidant activity, the higher value was measured in both androgenic lines from variety “Slonovo uvo”.

**Table 1.** Morphological evaluation of fruit and productivity in androgenic lines and four parental genotypes.

| Line               | Fruits Characters   |                    |                   |                        |                    | Productivity/<br>Plant (g) | Fruit/<br>Plant No. |
|--------------------|---------------------|--------------------|-------------------|------------------------|--------------------|----------------------------|---------------------|
|                    | Weight<br>(g)       | Length<br>(cm)     | Width<br>(cm)     | Wall Thickness<br>(mm) | Usable Part<br>(g) |                            |                     |
| 211                | 141.3 <sup>a</sup>  | 14.0 <sup>ns</sup> | 6.2 <sup>ab</sup> | 6.5 <sup>a</sup>       | 124.5 <sup>a</sup> | 2047 <sup>a</sup>          | 15 <sup>ns</sup>    |
| 212                | 127.0 <sup>a</sup>  | 14.4 <sup>ns</sup> | 6.4 <sup>a</sup>  | 6.5 <sup>a</sup>       | 108.3 <sup>a</sup> | 1398 <sup>b</sup>          | 12 <sup>ns</sup>    |
| 213                | 125.8 <sup>a</sup>  | 15.1 <sup>ns</sup> | 6.6 <sup>a</sup>  | 6.5 <sup>a</sup>       | 110.1 <sup>a</sup> | 1449 <sup>b</sup>          | 12 <sup>ns</sup>    |
| F <sub>1</sub> 202 | 90.8 <sup>b</sup>   | 13.8 <sup>ns</sup> | 5.0 <sup>b</sup>  | 4.0 <sup>b</sup>       | 83.3 <sup>b</sup>  | 1150 <sup>b</sup>          | 12 <sup>ns</sup>    |
| 214                | 201.5 <sup>a</sup>  | 15.0 <sup>a</sup>  | 7.5 <sup>a</sup>  | 9.1 <sup>a</sup>       | 181.5 <sup>a</sup> | 2043 <sup>a</sup>          | 11 <sup>ns</sup>    |
| 215                | 146.0 <sup>b</sup>  | 13.0 <sup>b</sup>  | 6.5 <sup>b</sup>  | 5.1 <sup>b</sup>       | 130.5 <sup>b</sup> | 1414 <sup>b</sup>          | 11 <sup>ns</sup>    |
| S. uvo             | 105.6 <sup>b</sup>  | 13.6 <sup>ab</sup> | 6.4 <sup>b</sup>  | 5.5 <sup>b</sup>       | 92.0 <sup>b</sup>  | 1193 <sup>b</sup>          | 11 <sup>ns</sup>    |
| 216                | 100.1 <sup>ab</sup> | 11.9 <sup>ab</sup> | 5.1 <sup>a</sup>  | 5.5 <sup>ns</sup>      | 85.2 <sup>a</sup>  | 1166 <sup>ns</sup>         | 12 <sup>ab</sup>    |
| 217                | 86.3 <sup>bc</sup>  | 10.4 <sup>b</sup>  | 5.0 <sup>a</sup>  | 4.9 <sup>ns</sup>      | 74.6 <sup>ab</sup> | 1141 <sup>ns</sup>         | 13 <sup>ab</sup>    |
| 218                | 77.7 <sup>c</sup>   | 11.0 <sup>b</sup>  | 4.9 <sup>a</sup>  | 4.9 <sup>ns</sup>      | 64.2 <sup>b</sup>  | 1415 <sup>ns</sup>         | 18 <sup>a</sup>     |
| 219                | 86.1 <sup>bc</sup>  | 10.8 <sup>b</sup>  | 5.1 <sup>a</sup>  | 4.9 <sup>ns</sup>      | 71.3 <sup>ab</sup> | 1250 <sup>ns</sup>         | 15 <sup>ab</sup>    |
| 220                | 106.0 <sup>a</sup>  | 13.0 <sup>a</sup>  | 5.3 <sup>a</sup>  | 5.1 <sup>ns</sup>      | 89.0 <sup>a</sup>  | 1190 <sup>ns</sup>         | 12 <sup>b</sup>     |
| Stryama            | 76.4 <sup>c</sup>   | 11.5 <sup>ab</sup> | 4.5 <sup>b</sup>  | 4.0 <sup>ns</sup>      | 65.7 <sup>b</sup>  | 1193 <sup>ns</sup>         | 17 <sup>a</sup>     |
| 221                | 70.7 <sup>ns</sup>  | 12.1 <sup>ns</sup> | 4.4 <sup>ns</sup> | 4.7 <sup>a</sup>       | 59.4 <sup>ns</sup> | 1237 <sup>ab</sup>         | 17 <sup>ab</sup>    |
| 222                | 85.7 <sup>ns</sup>  | 13.7 <sup>ns</sup> | 4.8 <sup>ns</sup> | 4.7 <sup>a</sup>       | 72.4 <sup>ns</sup> | 1105 <sup>b</sup>          | 14 <sup>b</sup>     |
| 223                | 80.4 <sup>ns</sup>  | 13.4 <sup>ns</sup> | 4.8 <sup>ns</sup> | 4.0 <sup>ab</sup>      | 68.4 <sup>ns</sup> | 1296 <sup>ab</sup>         | 17 <sup>ab</sup>    |
| 224                | 80.3 <sup>ns</sup>  | 12.1 <sup>ns</sup> | 4.9 <sup>ns</sup> | 4.0 <sup>ab</sup>      | 70.2 <sup>ns</sup> | 1418 <sup>a</sup>          | 18 <sup>a</sup>     |
| Z. medal           | 77.8 <sup>ns</sup>  | 14.3 <sup>ns</sup> | 4.7 <sup>ns</sup> | 3.0 <sup>b</sup>       | 67.1 <sup>ns</sup> | 1303 <sup>ab</sup>         | 17 <sup>ab</sup>    |

a–c:  $p \leq 0.05$ , Duncan’s Multiple Range Test; ns: not significant.

Among androgenic lines originated from variety “Stryama”, the highest fruit weight, length and width were recorded in line “220” followed by line “216”. The Vit C was found highly variable among androgenic lines and donor genotype “Stryama”. The highest Vit C content was recorded in line “218” (70.2 ng/100 g fresh weight (FW)), while the least in line “220” (39.2 ng/100 g FW). Among the androgenic lines, no significant differences in DM and TP content were seen, but the measured values were higher than those in the control. The FRAP antioxidant activity varied from 6.0  $\mu\text{mol Fe}^{2+}/\text{g FW}$  (line “220”) to 8.7

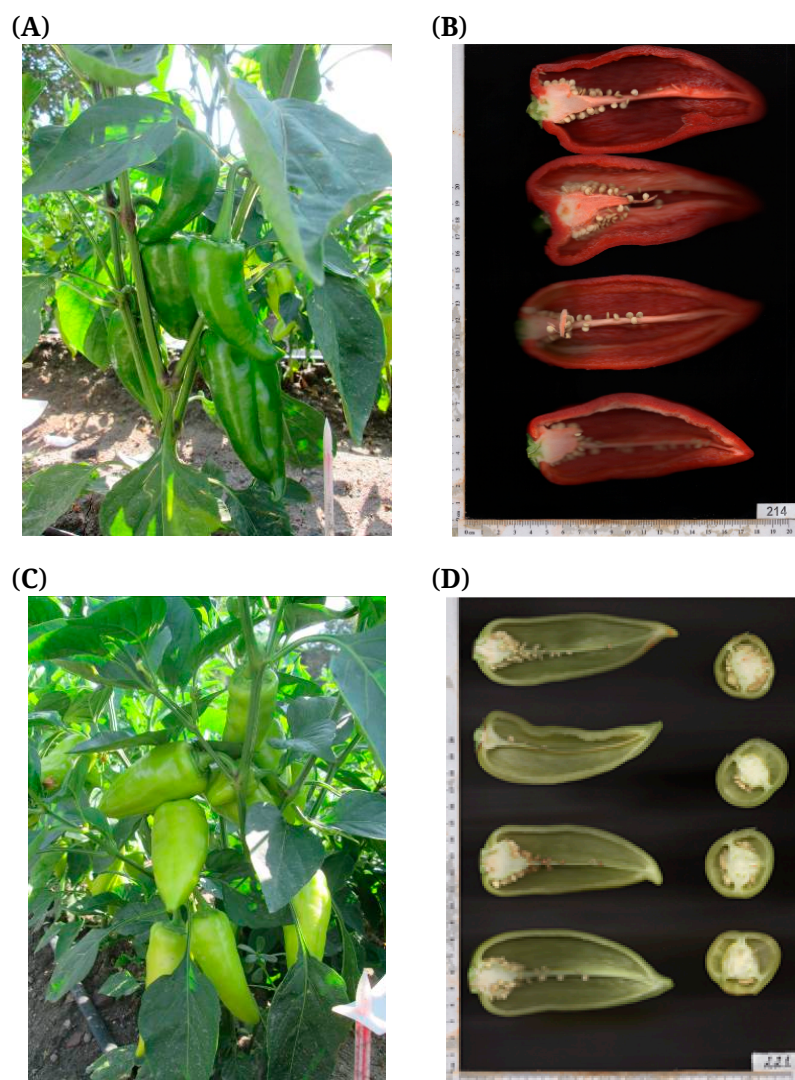
$\mu\text{mol Fe}^{2+}/\text{g FW}$  (line “216”) and 4.5,  $\mu\text{mol Fe}^{2+}/\text{g FW}$  for variety “Stryama”.

**Table 2.** Evaluation of same fruit quality traits and dry matter content in androgenic pepper lines and parental genotypes.

| Line               | Vit C,<br>ng/100 g FW | Dry matter,<br>% | Total Polyphenols,<br>mg GAE/100 g FW | FRAP,<br>$\mu\text{mol Fe}^{2+}/\text{g FW}$ |
|--------------------|-----------------------|------------------|---------------------------------------|--|
| 211                | 190.1                 | 10.83            | 143.8                                 | 13.5   |
| 212                | 167.8                 | 10.32            | 154.5                                 | 11.9   |
| 213                | 136.1                 | 10.59            | 132.8                                 | 8.3  |
| F <sub>1</sub> 202 | -                     | -                | -                                     | -  |
| 214                | 192.0                 | 11.73            | 193.0                                 | 12.1   |
| 215                | 237.7                 | 12.17            | 178.6                                 | 15.9   |
| S. uvo             | 151.9                 | 11.28            | 153.6                                 | 8.0  |
| 216                | 40.8                  | 7.38             | 150.1                                 | 8.7  |
| 217                | 55.5                  | 7.36             | 148.5                                 | 7.8  |
| 218                | 70.2                  | 7.90             | 151.6                                 | 6.9  |
| 219                | 35.9                  | 7.50             | 120.4                                 | 6.6  |
| 220                | 21.2                  | 7.06             | 131.3                                 | 6.0  |
| Stryama            | 39.2                  | 6.05             | 105.2                                 | 4.5  |
| 221                | 106.1                 | 7.59             | 77.6                                  | 4.6  |
| 222                | 52.2                  | 7.41             | 62.8                                  | 3.7  |
| 223                | 84.9                  | 7.25             | 73.4                                  | 4.3  |
| 224                | 106.1                 | 8.03             | 85.4                                  | 5.5  |
| Z. medal           | 24.2                  | 7.23             | 86.4                                  | 3.2  |

The lines originated from variety “Zlaten medal 7” did not show higher values of the fruit morphology traits, but lines “221” and “224” showed 2.5-fold increase in Vit C content (Figure 1C,D). Only line “224” distinguished by the DM with the value over 8.0%. Data showed that the TP content was higher in the donor genotype “Zlaten medal 7”. Highest FRAP antioxidant activity of 5.5  $\mu\text{mol Fe}^{2+}/\text{g FW}$  was recorded in line “224”, followed by line “221” 4.6  $\mu\text{mol Fe}^{2+}/\text{g FW}$  least was reported in donor genotype “Zlaten medal 7”.

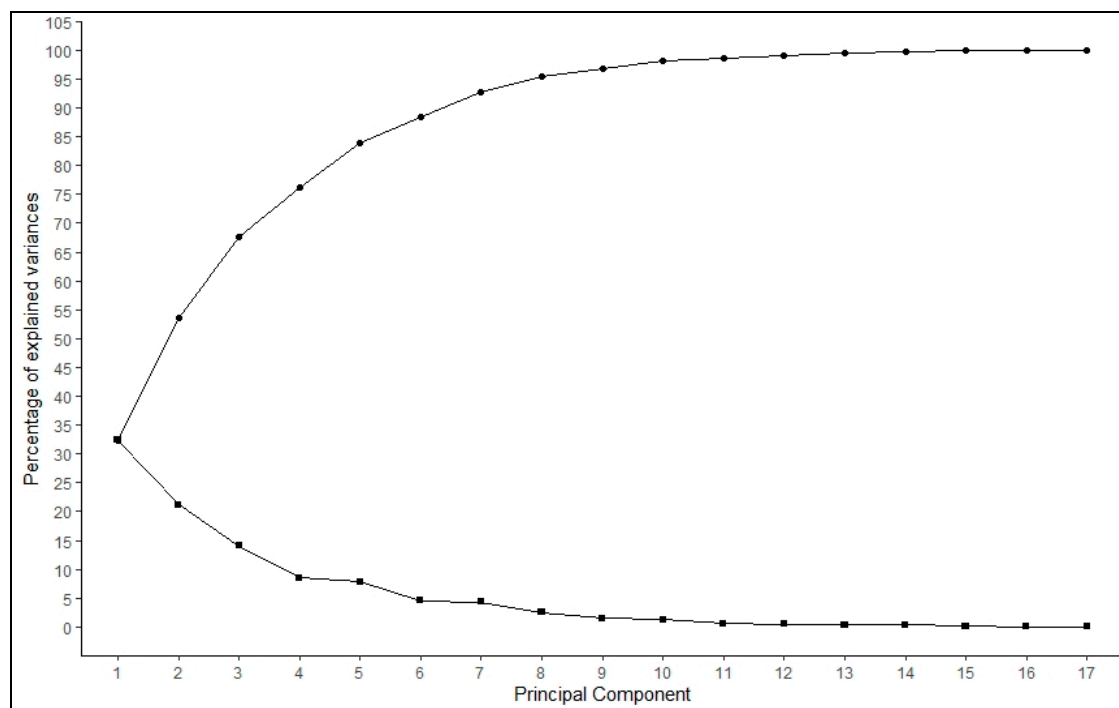
Multivariate Principal component analysis (PCA) was employed to detect and identify the trait combination that most contributed to the total cumulative variation. A total of 17 principal components were identified during PCA (Figure 2); however, we utilized factor analysis to identify those principal components that had an eigen value of >1 (Table 3) and eight major PCs explained around 95.4% variation to the total variation. The TA descriptors individual PC1 to PC8 contributed 32.4%, 21.1%, 14.1%, 8.5%, 7.8%, 4.6%, 4.4%, and 2.5% variation, respectively (Figure 2 and Table 3).



**Figure 1.** Androgenic pepper plants (A) Pepper plant from line “214” (B) Scanned fruits from line “214” (C) Pepper plant from line “221” (D) Scanned fruits from line “221”.

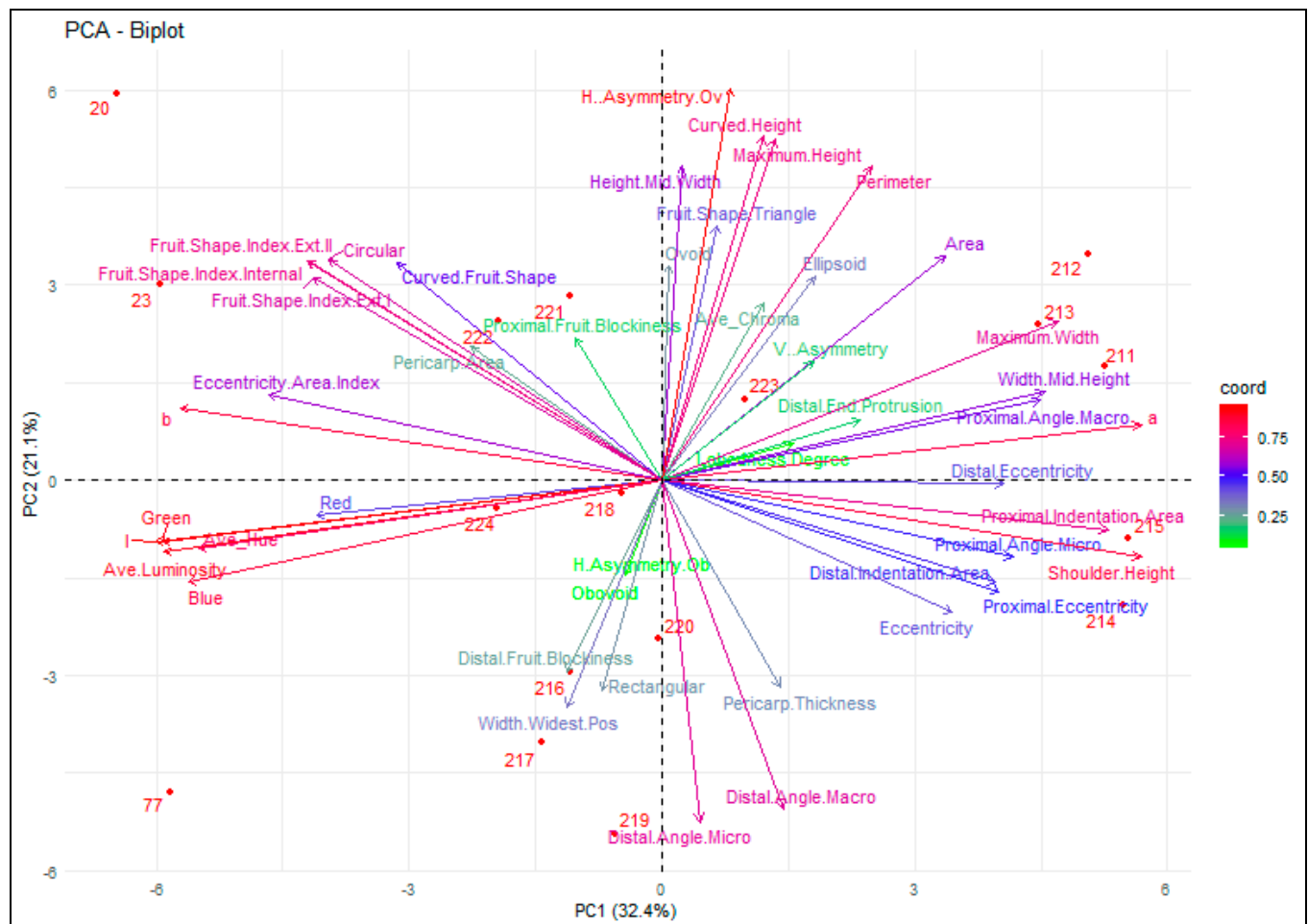
**Table 3.** Eigenvalues, % variance, and % cumulative variance as explained by extracted factors in factor analysis.

| Component | Eigan Values | % Variance | % Cumulative Variance |
|-----------|--------------|------------|-----------------------|
| 1         | 15.2         | 32.4       | 32.4                  |
| 2         | 9.9          | 21.1       | 53.5                  |
| 3         | 6.6          | 14.1       | 67.6                  |
| 4         | 4.0          | 8.5        | 76.1                  |
| 5         | 3.7          | 7.8        | 83.9                  |
| 6         | 2.2          | 4.6        | 88.5                  |
| 7         | 2.0          | 4.4        | 92.9                  |
| 8         | 1.2          | 2.5        | 95.4                  |



**Figure 2.** PCA variance plot displaying percent variation contributed by each principle component. The line with circle indicates the cumulative variation of 1–17 components, and the line with square indicates variation explained by an individual principle component.

The PCA accession and feature biplot displayed that all DH lines were spread across all four quadrants (Figure 3); however, DH lines derived from a specific variety populated in specific quadrants. Double haploid lines “221”, “222” derived from “Zlaten medal 7” as well as parental genotypes of 20 (“Zlaten medal 7”) were limited to quadrant 2 (negative quadrant of PC1 and positive quadrant of PC2), while DH lines “223” and “224” were scattered in quadrant 1 (positive quadrant of PC1 and PC2), and quadrant 3 (negative quadrant of PC1 and PC2), respectively. Double haploid lines “211”, “212”, “213” derived from the F<sub>1</sub> hybrid “202” were distinctly separated in quadrant 1 (Figure 3). Double haploid lines “214” and “215” derived from “Slonovo uvo” populated in the positive quadrant of PC1 and negative quadrant of PC2 (quadrant 4), whereas parental genotype of 47 (“Slonovo uvo”) was populated in quadrant 3. Double haploid lines “216”, “217”, “218”, and “219” derived from “Stryama” were populated in quadrant 3 while parental genotype 23 (“Stryama”) was located in quadrant 2. PC1 exhibited contribution by color, proximal fruit end shape, internal eccentricity whereas fruit size, fruit blockiness, and distal fruit end shape descriptors contributed to PC2 (Figure 3).



**Figure 3.** The relationship of the first (PC1) and second (PC2) components in the analysis of 14 pepper DH lines and three parental genotypes.

## DISCUSSION

In recent years, the application of anther culture as a breeding method has significantly increased due to its advantage in speeding up the breeding process. By anther and pollen culture, DH plants can be produced within a year in comparison to prolong inbreeding, which usually takes more than six years.

Pepper belongs to a recalcitrant species characterized by low frequency of embryo induction from anthers and low rate of subsequent conversion to normal plant-regenerants [2,20]. Despite this difficulty, the number of studies concerning the practical application in different *Capsicum* species has steadily increased in recent years [21–25]. Different studies indicate that positive changes in plant productivity, fruit morphology, resistance to viruses and pests can be induced with anther culture [26–28]. The findings from the present study showed significant variation for main fruit characters and productivity among androgenic lines of same origin. These differences may be due to the somatic changes that occur in the pollen grain DNA, which likely results into new gene combinations obtained during meiosis [10,29]. The phenotypic diversity

reflects the genetic variability of microspores they originated from and essential characteristics of androgenic populations, which greatly facilitates the selection of plants valuable for breeders [8,29]. Nowaczyk et al. [30] indicated that 74% of studied androgenic plants were different compared to the donor genotype for ripe fruit color, pericarp thickness, and fruit taste. Other nine diploid androgenic plants showed differences from the mother genotypes for fruit weight, fruit wall thickness, and dry matter content. Other investigations also reported variation in plant and fruits characters between androgenic lines of the same origin [8,29]. Shrestha et al. [29] suggested that the differences between DH lines may be due to the results of naturally produced variation, mutation during anther culture, but also has been attributed to residual heterozygosity in a donor plants for anther culture.

In the current study, androgenic lines derived from hybrid “202” and “Slonovo uvo” were distinguished with the heaviest fruits compared to the parental genotypes. Average fruit weight was also higher in the DH lines originated from varieties “Stryama” and “Zlaten medal 7”, but the differences were not substantial. Fruit wall thickness was the trait by which all DH lines were superior to the parental genotypes. Moreover, our data showed higher productivity per plant in six DH lines compared to the donor genotypes. Similar results with improved fruit morphology characteristics were obtained in other studies [8,12,13]. On the other hand, Luitel et al. [31] reported that plant and fruit characters in DH plants were lower than the standard varieties but should not be omitted in the process of DHs evaluation. After detailed study of agromorphological and molecular traits in eight double haploid pepper lines, it was established that two of them had higher yield [32]. As pointed out in different investigations, DH lines can be used as a tool for enrichment of biodiversity and fast development of valuable pepper genotypes for future breeding [11,13].

Pepper fruits are rich in biologically active substances such as carotenoids, vitamins, flavonoids [33]. However, the variation of the fruit biochemical composition is mainly affected by the genotype [34]. The results of the current study showed a wide variation in Vit C, dry matter content, TA, and FRAP among studied DH lines. Generally, the highest value of Vit C content was obtained in red colored fruits. Nevertheless, in lines “221” and “224” the Vit C content was 2.5 fold higher than the control “Zlaten medal 7”. Luitel and Kang [11] observed the higher value of Vit C in yellow and orange colored fruits compared to red genotypes. The authors suggested that the variation could be due to the genotype, maturity stage, and environmental conditions. The variation in dry matter content among the studied DH lines and initial genotypes was variable from 6.05% to 12.17%. The same results with variation in dry matter content in different lines with androgenic origin between 7.85% and 13.0% was also reported [8]. The highest value of total polyphenols was measured in line “214” (193.0 mg GAE/100 g FW) and the least in line

“222” (62.8 mg GAE/100 g FW). In contrast, Luitel and Kang [11] reported the highest content of gallic acid of 18.8 mg g<sup>-1</sup> of FW in androgenic line MY-3. In all studied DH lines, antioxidant activity was higher than the initial genotypes and varied widely. The results of the present study are in accordance with previous research, which indicated that antioxidant activity varied among pepper varieties and DH lines [11,35]. Furthermore, higher polyphenol content positively correlated with antioxidant activity [35].

## CONCLUSIONS

The results of the present study showed that androgenic pepper lines were different from the parental genotype. Variation among androgenic lines of same origin also was observed. On the basis of morphological and fruit quality evaluation, pepper lines “211”, “214”, “215” and “224”, superior to the parental genotypes, were selected and will be further utilized in future breeding programs. Findings of this study demonstrate that the application of anther culture can assist in obtaining genetic variation and transgressive traits. The accessible genetic diversity of pepper can be resourceful in development of novel breeding lines with improved quantitative and qualitative fruit quality traits.

## AUTHOR CONTRIBUTIONS

SG designed the manuscript. SG, IT, AN and DK carried out the experimental work. SG and IT analyzed the results. AN designed the data of TA. VT provided the seeds from parental genotypes. DK supervised the process. All authors read and corrected the manuscript.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

## FUNDING

This study was funded by the National Science Fund of Bulgaria [Grant DN06/4] and the financial support by Horizon 2020 PlantaSYST project under Grant Agreement No 739582.

## ACKNOWLEDGMENTS

We would like to acknowledge the efforts of technical staff of Plant Tissue Culture Laboratory of Maritsa Vegetables Crops Research Institute (MVCRI) in conducting the experiments to generate the DH lines.

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How to cite this article:

Grozeva S, Tringovska I, Nankar AN, Todorova V, Kostova D. Assessment of Fruit Quality and Fruit Morphology in Androgenic Pepper Lines (*Capsicum annuum* L.). *Crop Breed Genet Genom*. 2020;2(1):e200005. <https://doi.org/10.20900/cbgg20200005>