

Evaluation and Agronomic Potential of Androgenic Pepper Genotypes Derived from Piran (*Capsicum annuum* L. cv. Piran)

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Authors' contributions

This work was carried out in collaboration between both authors. Author FT planned and carried out the experimental work, performed the statistical analysis, literature collection and manuscript writing. Author LKG was in charge of overall planning and supervision of the experiment and participated in manuscript preparation. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was to evaluate and estimate the agronomic potential of two androgenic genotypes via important plant, fruit and production traits compared to the mother genotype Piran.

Place and Duration of Study: Strumica, Faculty of Agriculture, Goce Delcev University-Stip, Republic of Macedonia, four-years experiment.

Methodology: The experiment was set up in random block design in four replications. Evaluation of the androgenic genotypes was performed according to morphological traits given in Descriptor for *Capsicum* spp. The analysis of plant traits was conducted on 20 randomly chosen plants (5 plants per replication) in maximum growth stage from each androgenic genotype and the control Piran. The analysis of fruit traits was conducted on 40 randomly chosen fruits (10 fruits per replication) in ripe stage from each androgenic genotype and the control.

Results: Androgenic genotype P3 was characterized as tall pepper type with medium-thick stem and large leaves. According to the fruit weight (41.45 g) and the fruit index higher than 4, the

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genotype is classified as long, horn-shaped, middle-size fruit pepper. The fruit flesh of 77.05% and the pericarp thickness 0.26 cm characterized this fruit as medium-fleshy. Androgenic genotype P4 was characterized as very tall pepper type with medium-thick stem and medium-large leaves. The fruit weight was 42.84 g, contained averagely 7.37% dry matter, 76.71% fruit flesh and 0.25 cm pericarp thickness. P4 fruit was long, horn-shaped, medium-size and medium-fleshy. Fruits of both genotypes were suitable for fresh and preserved consumption.

Conclusion: Analysis of morphological and productivity traits of androgenic genotypes provides valuable information for future breeding. Androgenic genotype P3 differ from the initial genotype for four traits, while the P4 genotype for six traits. The results of this research prove the advantage of pepper androgenesis as a tool for enrichment of biodiversity and fast development of valuable pepper genotypes for future breeding.

Keywords: Androgenesis; pepper; Piran; androgenic genotype P3; androgenic genotype P4; morphological traits; characterization.

1. INTRODUCTION

Pepper (*Capsicum annuum* L.) is an important crop that can be improved by utilization of the methods of classical genetics, but also by using plant tissue culture and plant biotechnology tools which can shorten breeding process of many crops [1-2].

Androgenesis is one of the methods utilized for creation of haploid and spontaneous dihaploid plants in *in vitro* culture [3-5]. Androgenesis can be defined as a set of biological processes leading to an individual that genetically originated exclusively from a male nucleus. The main objective of haploid research is to obtain dihaploid homozygous lines with high practical value for breeders, but experiments in this area also have theoretical importance for fundamental biological sciences (genetics, embryology, cytology, and molecular biology). After spontaneous or induced genome doubling, dihaploids that are completely homozygous can be utilized differently in fundamental and paratactical research programs. From the standpoint of plant breeding, androgenesis has power to reduce the typical 7–9 inbreeding generations necessary to stabilize a hybrid genotype to only one. It is the key advantage of dihaploid technology in the context of plant breeding. Within this same context of plant breeding, dihaploids, which are homozygous for all of their loci, create a powerful tool for the study of the genetic basis of quantitative characteristics, including the genetic mapping of complex traits such as production or quality, the most agronomically interesting, and difficult to be addressed by other approaches [3].

There are reports about a high frequency of anther-derived embryos of different *Capsicum* species, but few of them developed into normal

plants. Despite the considerable number of studies conducted with pepper anther culture, the reported effectiveness of the process is still low. Additionally, pepper is characterized as third culture of fam. *Solanaceae* which is “reluctant” on androgenic methods, after tomato and eggplant [3]. Even there are androgenic embryoids, they either cannot be regenerated into fully developed plant or are infertile. Critical factors for production of haploid/dihaploid embryoid and their regeneration into fertile plants are growth conditions of donor-plants, selection of flower buds in appropriate microspore stadium, stress, medium composition, genetic background of mother genotypes and environmental culture conditions [4]. On the other hand, breeding programs require availability of a considerable number of genetically stable plants. In this regard, the most important achievement is development and optimization of protocols for haploid and dihaploid production and regeneration of pepper under laboratory conditions and utilization of the novel di-haploid lines in practice [5].

Gained pepper haploids and dihaploids have certain genetic potentials which are quickly phenotypically expressed because they origin from a haploid cell [6-7]. These androgenic plants, before being included in breeding process, must be studied for their growth and development stages, their genetic and morphological characteristics compared to the mother genotype which give information about similarities and differences between androgenic and mother genotypes [8-10].

This study was conducted to evaluate and estimate the agronomic potential of two androgenic genotypes P3 and P4 derived from initial genotype Piran via important morphological and production traits.

2. MATERIALS AND METHODS

Detailed description of the methodology and the process of creation of androgenic genotypes is presented in different publications [11-14].

In this research plants and fruits of two androgenic genotypes P3 and P4 created from the variety Piran were tested in greenhouse conditions at the Faculty of Agriculture, Goce Delcev University – Strumica, Republic of Macedonia, during four-years experiment. The variety Piran, used as mother genotype in anther culture, was used as control (standard) during evaluation of the androgenic genotypes P3 and P4. It was obtained from the gene bank of Faculty of Agriculture, Goce Delve University – Stip [15].

Piran is Macedonian variety which can be cultivated in open field and under protected conditions. The average plant height is 80 cm, fruits are large with average weight of 100-120 g, yellow in unripe stage and red when ripe. Piran average yield is 60-80 t/ha [14,16].

2.1 Research Design and Dynamics

During the first experimental year seeds obtained from fertile androgenic plants were utilized as starting material in the experiment. Seeds from selected typical fruit from each androgenic genotype of the previous experimental year were used in the subsequent experimental years.

In each experimental year seeds of studied genotypes were sown in the beginning of April in non-heated greenhouse. At the end of May, the seedlings were transplanted into pots (d=22 cm) filled with mixture of soil, sand and peat. The experiment was design in four replications, in random block design. From each androgenic genotype and control genotype Piran, 40 plants (10 per replication) were transplanted in plastic pots, which were placed in the greenhouse with 30cm/100cm distance scheme. Plants were cultivated according to standard cultivation practices under protected conditions. Irrigation and nutrition were performed as fertigation with drip system.

Selection of parameters and evaluation of genotypes was performed according to recommendations given in Descriptor for *Capsicum* spp. [17]. The analysis of plant traits was conducted on 20 randomly chosen plants (5 plants per replication) in maximum growth

stage of each androgenic genotype and the control Piran. The following plant traits were analyzed:

- Plant height (cm)
- Plant height to the first branching (cm)
- Stem thickness (cm)
- Internode length (cm)

The analysis of fruit traits was conducted on 40 randomly chosen fruits (10 per replication) in ripe stage from for each androgenic genotype and the control. The following fruit traits were analyzed:

- Fruit length (cm)
- Fruit width (cm)
- Fruit index (fruit shape)
- Fruit weight (g)
- Number of fruit locules
- Fruit flesh percentage (%)
- Pericarp thickness (cm)
- Dry matter in fresh fruit (%)

The analysis of productivity traits was conducted on whole experiment level. During the research the following productivity traits were analyzed:

- Number of fruits per plant
- Yield per plant (g)
- Total yield (kg/m²)

A classification of plant, leaf, fruits and productivity of androgenic genotypes was made based on the results of studied morphological and production traits according to Jankuloski (1983, 1997). It is the most suitable and acceptable classification of peppers by morphological traits which are bred and cultivated in the Western Balkans [18-19].

2.2 Biometric Analysis of Characteristics

Statistical analysis of variance is applied for the evaluation of the experiment in general and each of the characteristics among all tested genotypes (One-Way ANOVA test).

For evaluation of the differences among examined genotypes Duncan's multiple range test is utilized for each trait in each experimental year on level 0.05% and for the four-year average on level 0.05% and 0.01%.

The statistical analyzation of the results was performed by IBM SPSS Statistics Software 19.0.

3. RESULTS AND DISCUSSION

3.1 Morphological Traits of Androgenic Plants

Morphological traits of pepper plants as total plant height and plant height to the first branching, leaf shape, stem thickness, fruit size and shape are traits which give basic marks of a pepper variety. However, they are dependent on many external factors as temperature, air humidity and cultivation practices [1,14]. The mean values of morphological traits of plants in maximum growth stage during the four-years research of androgenic lines P3, P4 and the mother genotype Piran are presented in Table 1.

The average plant height was 87.61 cm (Piran), 88.55 cm (P3 genotype) and 93.68 cm (P4 genotype). In I and II research year, the plant height showed significant difference between P4 genotype and the control, while in IV year, the plant height of P3 and P4 was significantly different as compared to the control. The average height to the first branching of plant was 39.48 cm (P3) and 39.48 (P4) and it was significantly different as compared to the control genotype (34.50 cm). For the first three experimental years, there was a significant difference between the mean values for P3 and P4 genotypes and the control Piran. Other researchers also reported differences in plant height between androgenic lines and initial variety [20-22]. It was found that all anther-derived lines were with higher values of plant height compared to the initial variety Hebar [20]. Diploid plants derived from minipaprika red showed more variation in plant height than the plants of minipaprika yellow and orange.

Similarly, researchers observed variation in leaf size, petiole and internode length, flower bud size, nodal color, leaf color, and leaf within the diploid lines of each minipaprika [21]. Differences by plant traits between anther-derived genotypes and the initial variety were observed in the field conditions [22].

Stem thickness of tested genotypes varied from 0.65 cm (P3, I year) to 1.14 cm (P4, I year), where the lowest average value was measured in IV experimental year and the highest in III experimental year. Averagely for the whole experimental period, the highest average value for stem thickness was registered for P4 genotype (1.01 cm) and it was significantly different from the control Piran (0.89 cm).

The mean internode length of studied genotypes has not shown statistically significant difference. Longer internodes were measured during the first two experimental years as compared to internode length in the third and the fourth experimental year. Many plants, including pepper, have internodes length which is determined by day-night temperature variations, which is mainly due to increased cell elongation than due to increasing of cell number. Peppers have shorter internodes when difference between day and night temperatures is smaller [23]. Morphological characterization of anther derived plants in minipaprika showed variation in leaf size, petiole and internode length, flower bud size, nodal color, leaf color, and leaf shape of diploid lines of each minipaprika. As expected, the researchers recorded reduced plant height, leaf size and internodes for haploid regenerants [21,24].



Fig. 1. Plants and fruits of variety Piran used as control genotype in the experiment

Table 1. Morphological characteristics of plants at maximum growth stage of P3 and P4 androgenic genotypes and the control Piran

Experimental year	Genotype	Plant height (cm)	Height to the first branching (cm)	Stem thickness (cm)	Internode length (cm)
I	Piran	85.10b	33.80b	0.88b	9.90a
	P3	90.15b	40.73a	0.96ab	9.42a
	P4	98.15a	41.15a	1.14a	9.18a
II	Piran	93.60b	37.20b	0.90b	10.47a
	P3	99.30b	44.90a	0.95ab	9.50a
	P4	108.20a	45.20a	1.00a	10.10a
III	Piran	87.80a	28.10b	0.96a	4.45a
	P3	87.50a	32.45a	1.01a	4.78a
	P4	88.50a	34.30a	0.99a	4.92a
IV	Piran	79.83a	41.83a	0.76a	5.81a
	P3	63.44b	33.67b	0.65a	5.51a
	P4	63.00b	34.56b	0.75a	4.67a
Average I-IV	Piran	87.61 ^{a,1}	34.50 ^{b,1}	0.89 ^{b,1}	7.86 ^{a,1}
Average I-IV	P3	88.55 ^{a,1}	38.62 ^{a,2}	0.94 ^{ab,12}	7.59 ^{a,1}
Average I-IV	P4	93.68 ^{a,1}	39.48 ^{c,2}	1.01 ^{a,2}	7.63 ^{a,1}

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $P=0.05$ and values followed by the same number do not differ significantly at $P<0.01$

Fig. 1 presents mean values for total plant height and plant height to the first branching during whole experiment in flowering stage and maximum growth stage of plants. The analysis of plant growth of the whole plant shows that the P3 plants, averagely have grown in height for 19.13 cm, P4 plants 21.91 cm, while the control plants 28.31 cm. This finding was an indicator that the mother variety Piran is characterized by the greatest potential of growth and development as compared to the androgenic genotypes. It can be considered as positive, if the aim of breeders is to gain taller plants, or negative if the aim is creation of shorter plants because of certain cultivation measures. On the other hand, the analysis of the plant height to the first branching showed that the average values of this trait between the two growth stages was the same as 3.25 (control Piran), 3.91 cm (P3 genotype) and 3.81 cm (P4 genotype) beside the difference of the total plant height of the genotypes analyzed.

The results of total plant height and plant height to the first branching show that plant growth between the flowering phase and maximum growth phase is happening above the first branching, consequently the plant grows in height with development and growth of new branches. The growth in stem base is very small. This characteristic is very important for cultivation of different pepper varieties under protected conditions because a lush growth will need stem and branches staking in order they to sustain fruit

load [14]. This is the reason for development of different systems for pepper plant support with temporary or permanent staking construction [25].

The average leaf length of androgenic genotypes was 11.15 cm (P3) and 10.73 (P4). The only statistically proven difference in leaf length was in II experimental year, when the leaf length of P3 genotypes (11.09 cm) was longer as compared to P4 genotype (11.09 cm) and the control Piran (10.21 cm). The leaves of P3 (5.41 cm) and P4 (5.22 cm) were significantly narrower than the control leaves (5.76 cm) which is proven during the whole experimental period. The average leaf index for androgenic genotypes P3 and P4 is 2.07, which was significantly higher than the control Piran (1.88). Leaves of P3 genotype were classified as large-size leaves, while P4 as medium-size leaves. Although they have the same leaf index, the first one were longer.

Other researchers presented results of leaves dimensions of diploid androgenic plants smaller than the mother genotype which is opposite to our results [24,26].

3.2 Morphological Traits of Fruits from Androgenic Plants

Morphological traits of pepper fruit vary in wide ranges. The most authors agree that variation

degree of morphological trait is genetically dependent and inherited, but they are always influenced by the complex of external factor, cultivation technology and practices, under protected conditions or open field conditions [18,25]. When making pepper characterization it is obvious that the most variations are observed in fruits, an organ which determines taxonomy and variety of the species [1,6,14,20,27,28]. Fruit size and shape, pericarp thickness and yield fruit are quantitative traits which are governed by polygenes, as well influenced by the environment. Yet, these are important plant characters that should not be omitted

in the process of evaluation of pepper dihaploids [21].

The average values for fruit length of androgenic genotypes and the control in the experimental period did not show significant difference for $P=0.05$ and $P<0.01$, which indicates that the control and androgenic genotypes are characterized by stability and good equability of fruit length (Table 3). Fruit length have not shown significant difference during I and II experimental year. In III experimental year the fruit length of the control genotype is significantly different compared to P4 androgenic genotype, while in

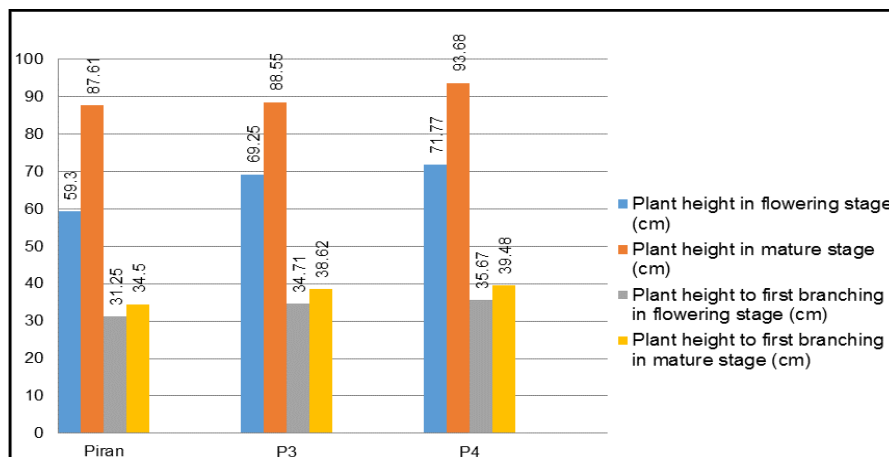


Fig. 2. Comparison of plant height and plant height to first branching in flowering stage and maximum growth stage of androgenic genotypes P3 and P4 and the control Piran

Table 2. Length, width and index of leaf of P3 and P4 androgenic genotypes and the control Piran

Experimental year	Genotype	Leaf length (cm)	Leaf width (cm)	Leaf index
I	Piran	11.86a	6.40a	1.86b
	P3	11.94a	5.89b	2.03a
	P4	11.31a	5.96b	2.03a
II	Piran	10.21b	5.33a	1.92b
	P3	11.09a	5.20ab	2.14a
	P4	10.32b	4.86b	2.14a
III	Piran	9.24b	5.04a	1.84b
	P3	10.41a	5.20a	2.01a
	P4	10.62a	5.09a	2.10a
IV	Piran	8.68a	4.45ab	1.96b
	P3	9.43a	4.24b	2.26a
	P4	9.25a	4.67a	1.96ab
Average I-IV	Piran	10.76 ^{a,1}	5.76 ^{a,2}	1.88 ^{b,1}
Average I-IV	P3	11.15 ^{a,1}	5.41 ^{b,12}	2.07 ^{a,2}
Average I-IV	P4	10.73 ^{a,1}	5.22 ^{b,1}	2.07 ^{a,2}

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $P=0.05$ and values followed by the same number do not differ significantly at $P<0.01$

Table 3. Length, width, index, weight and number of locules of ripen fruits of androgenic lines P3 and P4 and the control Piran

Experimental year	Genotype	Fruit length (cm)	Fruit width (cm)	Fruit index	Fruit weight (g)	Number of fruit locules
I	Piran	13.54a	3.37a	4.04a	32.17b	2.10a
	P3	14.41a	3.40a	4.28a	43.40a	2.00a
	P4	14.70a	3.50a	4.27a	48.00a	2.05a
II	Piran	13.31a	3.40a	3.96a	35.77b	2.30a
	P3	12.97a	3.51a	3.81a	39.59ab	2.55a
	P4	13.82a	3.63a	3.89a	43.79a	2.20a
III	Piran	12.88b	3.43b	3.79a	37.13b	2.10a
	P3	13.74b	3.66ab	3.79a	44.30a	2.15a
	P4	15.41a	3.78a	4.09a	50.10a	2.20a
IV	Piran	14.12a	3.09b	4.61a	35.06ab	2.30a
	P3	12.50b	3.37a	4.17ab	37.55a	2.07a
	P4	13.75a	3.18a	3.95b	29.48b	2.35a
Average I-IV	Piran	13.46 ^{a,1}	3.32 ^{b,1}	4.10 ^{a,1}	35.03 ^{b,1}	2.20 ^{a,1}
Average I-IV	P3	13.71 ^{a,1}	3.49 ^{a,1}	3.97 ^{a,1}	41.45 ^{a,2}	2.20 ^{a,1}
Average I-IV	P4	14.11 ^{a,1}	3.52 ^{a,1}	4.05 ^{a,1}	42.84 ^{a,2}	2.20 ^{a,1}

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $P=0.05$ and values followed by the same number do not differ significantly at $P<0.01$

IV experimental year there is significant difference between the control and P4 genotype. The biggest average fruit length is gained in the control fruits in III experimental year (15.41 cm), the smallest in P3 (12.50 cm) in IV. The fruit length during the experimental period vary from 10.46 cm to 14.11 cm. Other researchers reported fruit length from 12.7 cm to 20.7 cm for Piran androgenic lines grown in plastic tunnel [28].

The mean values of fruit width show that androgenic genotypes P3 (3.49 cm) and P4 (3.52 cm) were significantly different compared to the control (3.32 cm) for $P=0.05$. The fruit width of the control peppers showed variation of the average during the experimental years. The biggest width was measured for P3 (3.66 cm) in III experimental year, while the smallest for control (3.09 cm) in IV experimental year. The average of fruit index values varied from 3.79 to 4.61. Only in IV experimental year, the fruit index of androgenic genotype P4 (3.95) was significantly different than the control (4.61). The statistical analysis of fruit index for four-years experimental period has not shown statistical difference on both statistical levels.

The biggest mean fruit weight was recorded for P4 (42.84 g) compared to P3 (41.45 g) and the control (35.03 g). The fruit weight showed significant variation in the three experimental years, when the fruit weight was always

statistically heavier compared to the control fruits.

The average number of fruit locules was from 2.0 to 2.55 without statistically significant difference.

The four-year mean values of traits fruit flesh, pericarp thickness and dry matter in fresh fruit for androgenic lines P3, P4 did not differ significantly from the control Piran (Table 4). The highest mean percentage of fruit flesh was 83.54% (P4; I year) and the lowest for 72.26% (P3; III year). The thickest fruit pericarp was recorded in the last experimental year. The percentage of dry matter in fresh fruit varied during the whole experimental period. During II, III and IV experimental year, the values for this characteristic significantly differed among examined genotypes and the content of dry matter was lower for androgenic fruits as compared to the control fruits.

Other authors reported a genetic analysis of anther culture-derived diploids of *Capsicum* spp. which showed that seventy-four percent of studied androgenic plants differ from the donor plant phenotype in ripe fruit color, pericarp structure and taste. Other nine diploid androgenic plants showed differences from the mother genotypes in fruit weight, wall thickness and dry matter content. In the study, statistical analysis of seven valuable traits (fruit length, width, weight, pericarp weight, pericarp portion of

fruit weight, wall thickness, dry matter content) confirmed the significant differences between all the plants studied and their donor plants. The authors concluded that recombined microspores and not somatic cells of anthers were the origin of these diploids, thus they proved the androgenic origin of diploid plants [6]. Higher visual and taste quality, fruit firmness, dry matter content, total soluble content, titratable acidity, phenolic content, and antioxidant activity were exhibited in pepper dihaploid fruits [24]. In regards to fruit characteristics of androgenic lines, there is a report for selection of 5 superior dihaploid lines and observation of considerable variation in plant and fruit traits among other lines [26]. Other research group reported androgenic pepper lines of R2 progeny with improved yield characteristics and dry matter content in fruits [27]. Androgenic lines with positive traits were found after detailed characterization of morphological and production parameters of androgenic pepper fruits [28].

The results of androgenic lines study showed that they were characterized with uniformity and higher values of fruit length, fruit weight and wall thickness compared to the initial mother variety Hebar [20]. Other researchers found that diploid androgenic plants were characterized by big size fruit with high individual fruit weight and volume. Based on analysis of diploid populations in comparison to control variety Boogie, they selected some elite diploid lines for development

of sweet pepper varieties. Similarly, it was suggested that existing variation within diploid population might be due to the anther culture and it is a naturally produced variation [26]. Variation in plant and fruit characters was observed in anther derived spontaneous diploid population of minipaprika. The authors concluded that such variation in quantitative traits had an additive character. Nevertheless, they recorded lower mean values of fruit characters in androgenic diploid plants of each group than the standard varieties [21].

3.3 Production Traits of Androgenic Genotypes

The results which characterize production trait of pepper genotypes under this study are presented in Table 5. The average number of fruits per plant during the whole experimental period for androgenic genotypes P3 (5.65) and P4 (5.80) was smaller compared to the control genotype Piran (7.67 fruits). The fruit number per plant for androgenic genotypes varied from 3.25 to 9.68 and only in II experimental year they were significantly higher than the initial genotype Piran. The average yield per plant was 249.81 g (Piran), 222.73 g (P3) and 231.90 g (P4) without significant difference for $P < 0.01$. The highest yield per plant was 420.25 g (P4; III year), while the lowest was 97.5 g (P4; II year). The highest yield per plant for all genotypes under study was recorded during III experimental year.

Table 4. Fruit flesh, pericarp thickness and dry matter of ripen fruits of androgenic lines P3 and P4 and the control Piran

Experimental year	Genotype	Fruit flesh (%)	Pericarp thickness (cm)	Dry matter in fresh fruit (%)
I	Piran	81.83a	0.21a	8.98a
	P3	83.54a	0.22a	9.37a
	P4	81.73a	0.22a	9.31a
II	Piran	78.48a	0.17b	8.05a
	P3	75.86a	0.27a	7.95a
	P4	76.53a	0.29a	7.08b
III	Piran	73.22a	0.22a	7.45a
	P3	72.26a	0.24a	7.03ab
	P4	75.05a	0.23a	6.58b
IV	Piran	77.05a	0.35a	7.00a
	P3	76.37a	0.30ab	7.10a
	P4	73.54a	0.27b	6.53b
Average I-IV	Piran	77.65 ^{a,1}	0.23 ^{a,1}	7.87 ^{a,1}
Average I-IV	P3	77.05 ^{a,1}	0.26 ^{a,1}	7.91 ^{a,1}
Average I-IV	P4	76.71 ^{a,1}	0.25 ^{a,1}	7.37 ^{b,1}

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $P=0.05$ and values followed by the same number do not differ significantly at $P < 0.01$

Table 5. Productivity characteristics of androgenic genotypes P3 and P4 and the control Piran

Experimental year	Genotype	Number of fruits per plant	Yield per plant (g)	Total yield (kg/m ²)
I	Piran	8.05a	216.00a	1.350a
	P3	5.83a	218.13a	1.363a
	P4	5.25a	210.25a	1.314a
II	Piran	6.30a	204.5a	1.279a
	P3	4.70b	171.50a	1.072a
	P4	4.35b	166.00a	1.038a
III	Piran	10.50a	384.00a	2.400a
	P3	8.23a	361.25a	2.258a
	P4	9.68a	420.25a	2.627a
IV	Piran	5.85a	194.75a	1.215a
	P3	3.25a	112.50a	0.703ab
	P4	3.3a	97.50a	0.609b
Average I-IV	Piran	7.67 ^{a,1}	249.81 ^{a,1}	1.561 ^{a,1}
Average I-IV	P3	5.65 ^{a,1}	222.73 ^{a,1}	1.392 ^{a,1}
Average I-IV	P4	5.80 ^{a,1}	231.90 ^{a,1}	1.449 ^{a,1}

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $P=0.05$ and values followed by the same number do not differ significantly at $P<0.01$

The average yield for P3 was 1,392 kg/m² and 1.449 kg/m² for P4, while it was the highest for the control Piran (1,561 kg/m²). The total yield of P3 varied from 0.609 to 2.258 kg/m², while for P4 from 0.703 kg/m² to 2.627 kg/m². The control Piran yielded from 1.215 kg/m² to 2.400 kg/m² during different experimental years.

The total and standard yield of 6 androgenic lines was higher compared to initial variety Hebar and they surpassed the control by standard yield from 11.02% to 42.26% [20]. Eight double haploid lines of pepper were studied for agromorphological and molecular traits. Two of them derived a special attention regarding yielding because they were either at the level of the maternal form or surpassed both parental forms regarding yield level [29]. Eleven R2 generations under study differed considerably in yielding and fruit number. According to authors of this study, the high values of the coefficient of variation suggested that the yield and the number of fruit per plant depend significantly on the environmental conditions [30]. Finally, there was higher variation in fruit yield in spontaneous doubled haploids of minipaprika which is considered as androgenic variation under the nuclear control [21].

3.4 Agronomic Potential and Evaluation of Androgenic Genotypes

3.4.1 Androgenic genotype P3

Androgenic genotype P3 was created from the Macedonian variety Piran via androgenesis

in vitro conditions. In the phase of maximum growth, the plant reached height of 88.55 cm, while the mean stem height to the first branching was 44.29 cm with stem width of 0.94 cm. These traits classified P3 genotypes as tall pepper with medium-thick steam. The leaves were averagely 11.15 cm long and 5.41 cm width which characterized the genotype with large leaves. According to the fruit weight (41.45 g) and the fruit index higher than 4, the genotype is classified as long, horn-shaped, middle-size peppers. The fruit flesh of 77.05% and the pericarp thickness 0.26 cm characterize this fruit as medium-fleshy and suitable for consumption as fresh and preserved. Regarding productivity traits, this genotype was characterized by 5.65 fruits per plant, averagely weighting 222.73 g and total yield of 1.392 kg/m². According to production traits, in the given agroecological and cultivation conditions, it is a low-yield pepper genotype.

3.4.2 Androgenic genotype P4

Androgenic genotype P4 was created from the Macedonian variety Piran via androgenesis *in vitro* conditions. In the phase of maximum growth, the plant height was 93.68 cm, while the mean stem height to the first branching was 38.62 cm which classified it as very tall pepper. The stem width was 1.01 cm. The height and steam thickness classified this genotypes as very tall pepper with medium-thick steam. The leaf index, a ratio of mean leaf length (10.73 cm) and mean leaf width (5.22 cm), was 2.07 which classified the leaves as medium-large.



Fig. 3. Plants and fruits of androgenic genotype P3



Fig. 4. Plants and fruits of androgenic genotype P4

The fruit was characterized with weight of 42.84 g, contains averagely 7.37% dry matter, 76.71% fruit flesh and 0.25 cm pericarp thickness. P4 fruit is classified as long, horn-shaped, medium-size and medium-fleshy pepper. The total yield gained in this research was 1.449 kg/m². One plant was averagely loaded with 5.80 fruits weighting 231.90 g. According to production traits, in the given agroecological and cultivation conditions, it is a low-yield pepper genotype.

In regards to overall variability of traits of anther-derived plants, it is sourced from a genetic diversity of microspores which is an effect of random gene segregation during meiosis and our results agree with this finding. The existing difference within the diploid lines might be due to the result of naturally produced variation. Besides, most of the agronomic variation could originate from the changes that occur in the DNA of the vegetative pollen grain cell [31]. Phenotypic homogeneity of an androgenic population can point to the high level of its

homozygosity. Besides, due to a necessity of providing a biometrical description of the most important plant and fruit parameters, this method additionally allows for a simultaneous selection of the lines which present the characters of interest to breeders and for allocating them for further breeding work [27]. On the other hand, phenotypic variation of plant and fruit traits may be the result of environmental conditions, which may often modify plant morphology. It suggests that although the importance of biometrical characteristics of anther-derived lines is of great value, as it allows for an objective evaluation of the essential functional traits of plants, which is necessary for the selection of the most interesting materials for breeding programs, the biometrical analysis cannot be the main method for androgenic regenerant microspore origin evaluation. However, especially the total fruit yield, the weight and size of the fruit, as well as the pericarp thickness, appear to be the most important plant characters that should not be omitted while dihaploid lines are being evaluated [30].

4. CONCLUSION

Analysis of morphological and production traits of androgenic genotypes provides valuable information for future breeding. Androgenic genotype P3 differ from the control variety for the traits height to the first branching, leaf index, fruit length and fruit weight. Androgenic genotype P4 differ from the control variety for the traits height to the first branching, stem thickness, leaf index, fruit length, fruit weight and fruit dry matter content.

The most important application of anther-derived, androgenic cultures in pepper has been their use in breeding programs, finally aimed to produce commercial hybrids with maximum heterosis and new or improved traits. Our results prove the advantage of pepper androgenesis as a tool for enrichment of biodiversity and fast development of valuable pepper genotypes for future breeding. These androgenic genotypes are recommended for their inclusion in breeding programs for development of long sweet pepper varieties for fresh and processed consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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