



Assessment of Androgenic Genotypes Obtained from Sweet Pepper Varieties (*Capsicum annuum* L.)

Fidanka Trajkova^{1*} and Liljana Koleva Gudeva¹

¹*Faculty of Agriculture, Goce Delcev University – Stip, Krste Misirkov 10-A, 2000 Stip, Republic of Macedonia.*

Authors' contributions

This work was carried out in collaboration between both of the listed authors. Author FT planned and carried 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.

Article Information

DOI: 10.9734/JEAI/2017/37425

Editor(s):

- (1) Slawomir Borek, Department of Plant Physiology, Adam Mickiewicz University, Poland.
(2) Anita Biesiada, Department of Horticulture, Wrocław University of Environmental and Life Sciences, Poland.

Reviewers:

- (1) Godwin Michael Ubi, University of Calabar, Nigeria.
(2) Ivandilson Pessoa Pinto de Menezes, Instituto Federal Goiano, Brazil.

Complete Peer review History: <http://www.sciencedomain.org/review-history/21839>

Original Research Article

**Received 16th October 2017
Accepted 9th November 2017
Published 9th November 2017**

ABSTRACT

The aim of this study was to assess important characteristics of five androgenic genotypes through important fruit and production traits compared to the mother genotypes, Kurtovska Kapia and Feherozon. The experiment was conducted in complete randomized design in four replicates during four years experiment under glasshouse conditions. The assessment of the fruits and production traits of androgenic genotypes was performed according to instructions given in Descriptor for *Capsicum* spp. by IPGRI, AVRDC and CATIE. The fruits of androgenic genotypes KK1 and KK2 were characterized as long and triangular, large-size medium-fleshy pepper fruit. KK1 is evaluated as medium-yielding, KK2 as low-yielding genotype. Fruits of both genotypes are suitable for fresh consumption and processing. The fruits of F5 and F7 were evaluated as bell-shaped, large-size peppers with very thin pericarp, while F6 as blocky-elongated, large-size fruit. All three androgenic genotypes are estimated as medium-yielding.

*Corresponding author: E-mail: fidanka.trajkova@ugd.edu.mk;

Androgenic genotypes KK1 and KK2 differ from the control variety for four fruit traits. Androgenic genotypes F5, F6 and F7 differ from the control variety for six fruit traits. There was no variability of the productivity (yield) traits between studied androgenic genotypes and their parental genotypes. From the assessment of androgenic genotypes as compared to the control genotypes, Kurtovska Kapia and Feherozon, all five androgenic genotypes are recommended for future varietal development of sweet pepper varieties.

Keywords: Kurtovska Kapia; Feherozon; androgenesis; pepper; androgenic lines; fruit traits; production traits.

1. INTRODUCTION

Pepper (*Capsicum annum* L.) is one of the most important horticulture crops produced in open fields and greenhouse conditions, even under cold climate conditions in the northern hemisphere. The world top pepper producers are China, Mexico, Turkey, Indonesia and USA, while in Europe, Spain and Italy [1,2]. Beside its importance as food, lately pepper is considered also as functional food because of the high content of active biocomponents with positive health benefits [1,3]. Due to increased production and consumption of peppers, there is need for improvement of pepper diversity in terms of their appearance, taste and chemical composition. The improvement of pepper diversity is possible by engagement of the methods of classical breeding, but also by the means of plant biotechnology which can advance the breeding process. Hence, the method of androgenesis is one of biotechnological tools which is often exploited in different pepper pre-breeding programs [4-5]. During the past years, *in vitro* pepper androgenesis research is extended although its successful application depends on different external factors as pepper genotype, stage of microspores development and growth conditions of mother plants [4-6]. Still, there are reports on androgenic pepper lines with improved morphological and quality traits [7-8]. The breeding importance of androgenic lines can be determined on the basis of analysis of plant and fruit traits in successive generations. Several comprehensive researches were conducted for characterization of different traits of androgenic pepper genotypes to find valuable parameters for future pepper breeding [9-10].

The aim of this study was to assess important characteristics of five androgenic genotypes through important fruit and production traits when compared to the mother genotypes Kurtovska Kapia and Feherozon by four successive generations.

2. MATERIALS AND METHODS

The method of androgenesis was successfully applied for development of fertile androgenic plants *in vitro* conditions from the Macedonian variety Kurtovska Kapia and Hungarian variety Feherozon. Anther-donor plants were grown under greenhouse conditions. The flower buds were harvested when the corolla was of the same length as the calyx or slightly longer. The developmental stage of the microspores was determined in microscopic slides of acetocarmine squashes. Flower buds were surface sterilized in 70% ethanol for several seconds, then in 5% calcium hypochlorite $\text{Ca}(\text{ClO})_2$ + 2-3 drops Tween 20 for 10 minutes, and rinsed three times in sterile distilled water. After the removal of the filaments, anthers from three flower buds were placed in Petri dish (6 cm), with the concave face down, touching the culture medium. The method of Dumas de Valux et al. (1981) was used for induction of embryoid formation from pepper microspores [11]. The anthers were cultivated on CP medium + 0.01 mg/l KIN + 0.01 mg/l 2,4-D with incubation of eight days in darkness at $35 \pm 2^\circ\text{C}$, the following four days the anthers were transferred to clime chamber at $25 \pm 2^\circ\text{C}$ with photoperiod of 12 h light/12 h dark. Afterwards, the anthers were subcultured on R₁ medium + 0.01 mg/l KIN and placed in clime chamber at $25 \pm 2^\circ\text{C}$ with photoperiodic 12 h light/12h dark. Young embryoids emerging from the anthers were transferred onto hormone free V3 media for rooting. Plantlets were planted on sterile mixture of perlite : peat : sand (1:1:1) and acclimatized in clime chamber, and afterwards placed in greenhouse under cover in order to prevent crosspollination. The detailed description of the applied methodology and the process of creation of androgenic genotypes is presented in different publications [12-15].

In this research, two androgenic genotypes KK1 and KK2 created from the variety Kurtovska Kapia and fruits of three androgenic genotypes F5, F6 and F7 created from the variety

Feherozon were tested for their fruit characteristics in greenhouse conditions at the Faculty of Agriculture, Goce Delcev University – Strumica, Republic of Macedonia, during four-years experiment. The mother varieties genotypes Kurtovska Kapia and Feherozon, used as initial genotypes for androgenesis, were used as controls (standards) for comparison of the androgenic genotypes derived from them, respectively.

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 [10, 15].

In each experimental year, the 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 laid out in four replicates, in completely randomized design. From each androgenic genotype and control genotypes, 40 plantlets (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 [10,15].

Selection of parameters and assessment of genotypes was performed according to recommendations given in Descriptor for *Capsicum* spp. [16]. The analysis of fruit traits was conducted on 40 randomly chosen fruits (10 per replication) in ripe stage for each androgenic genotype and the control. During the research the following fruit traits were measured, recorded and 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, for each androgenic

genotype and the controls, respectively. 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 fruits and productivity of androgenic genotypes was made based on the results of studied morphological and production traits according to Jankuloski (1983, 1997) because it is the most suitable and acceptable classification of peppers by morphological traits which are bred and cultivated in the Western Balkans [17-18].

2.2 Biometric Analysis of Traits

The statistical analyses of the results were performed by IBM SPSS Statistics Software 19.0. 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 difference between examined genotypes, the Duncan's multiple range test was utilized for each trait in each experimental year on 0.05% and for the four-year average on level 0.05% and 0.01%.

3. RESULTS AND DISCUSSION

3.1 Assessment of Fruits Traits of Androgenic Genotypes

The assessment of fruit traits of Kurtovska Kapia androgenic genotypes and their mother genotype is presented in Table 1 and Table 2. The average fruit length was 10.69 cm (KKk), 10.46 cm (KK1) and 10.73 cm (KK2) in the whole experimental period without significant difference for $P<0.05$ and $P<0.01$, which indicates that the control and androgenic genotypes were characterized by stability and good equitability of fruit length. The mean values of fruit width of androgenic genotypes KK2 (5.45 cm) significantly ($p<0.05$) differed from KK1 (5.22 cm) for $P<0.05$. The biggest mean fruit index (2.07) was four-years experimental period was recorded for the fruits of control genotype Kurtovska Kapia and the lowest for the KK2 androgenic genotype (1.95). The biggest mean fruit weight was noted for KK2 (71.20 g) compared to the KK2 (65.05 g) and the control (64.74 g). The number of fruit locules mean values ranged from 2.12 (KKk and KK1) to 2.16 (KK2). Except number of fruit locules, the

other fruit traits showed significant variability ($p < 0.05$) through the consequent experimental years (Table 1).

The mean fruit flesh varied from 77.82% (KKk), 77.86% (KK1) to 70.06% (KK2) without significant difference ($p < 0.05$) during each experimental year and on whole experimental level. The highest average pericarp thickness was measured in androgenic line KK1 (0.37 cm) and the lowest in control genotype KKk (0.35

cm). The mean value of dry matter of fresh fruit varied from the lowest 6.65% (KK2) to the highest 7.34% (KK1), while it was 7.17% in control genotype. However, there was no recorded significant variability ($p < 0.05$) of these traits through the consequent experimental years (Table 2).

The fruit length of Feherozon derived genotypes showed great variation in all four experimental years. The average experimental values of fruit

Table 1. Assessment of fruit traits length, width, index, weight and number of locules of androgenic lines KK1 and KK2 and the control Kurtovska Kapia (KKk)

Experimental year	Genotype	Fruit length (cm)	Fruit width (cm)	Fruit index	Fruit weight (g)	Number of fruit locules
–	KKk	15.55a	4.82b	2.15a	54.81b	2.00a
	KK1	10.91a	5.46a	2.01a	73.33a	2.00a
	KK2	12.37a	5.59a	2.06a	79.68a	2.05a
=	KKk	10.26a	5.13a	2.00a	58.50a	2.10a
	KK1	9.94a	5.35a	1.88a	61.45a	2.15a
	KK2	10.44a	5.29a	1.99a	66.98a	2.05a
≡	KKk	12.27a	5.44a	2.25a	78.24a	2.10a
	KK1	10.58b	5.51a	1.93a	71.83a	2.05a
	KK2	10.67b	5.52a	1.94b	82.08a	2.25a
≥	KKk	9.60b	5.50a	1.75b	67.44a	2.30a
	KK1	10.41a	4.58c	2.29a	53.58b	2.30a
	KK2	9.27b	5.16b	1.80b	56.05b	2.25a
Average I-IV	KKk	10.69 ^{a,1}	5.26 ^{ab,1}	2.07 ^{a,1}	64.74 ^{b,1}	2.12 ^{a,1}
Average I-IV	KK1	10.46 ^{a,1}	5.22 ^{b,1}	2.03 ^{ab,1}	65.05 ^{b,1}	2.12 ^{a,1}
Average I-IV	KK2	10.73 ^{a,1}	5.45 ^{a,1}	1.95 ^{b,1}	71.20 ^{a,2}	2.16 ^{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 $P < 0.01$

Table 2. Assessment of fruit traits flash, pericarp thickness and dry matter of androgenic lines KK1 and KK2 and the control Kurtovska Kapia (KKk)

Experimental year	Genotype	Fruit flesh (%)	Pericarp thickness (cm)	Dry matter in fresh fruit (%)
–	KKk	81,34a	0,34a	7,84a
	KK1	85,19a	0,38a	8,44a
	KK2	85,17a	0,36a	7,92a
=	KKk	79,46a	0,32a	6,80a
	KK1	77,73a	0,34a	6,95a
	KK2	79,30a	0,31a	6,28a
≡	KKk	76,46a	0,34a	6,25a
	KK1	75,18a	0,35a	6,88a
	KK2	75,90a	0,34a	6,68a
≥	KKk	71,65a	0,43a	7,05a
	KK1	73,35a	0,41a	7,10a
	KK2	73,07a	0,41a	7,25a
Average I-IV	KKk	77,82 ^{a,1}	0,35 ^{ab,12}	7,17 ^{a,1}
Average I-IV	KK1	77,86 ^{a,1}	0,37 ^{a,1}	7,34 ^{a,1}
Average I-IV	KK2	79,06 ^{a,1}	0,36 ^{b,2}	6,95 ^{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 $P < 0.01$

length varied from the lowest 6.27 cm (F7) to the highest 9.40 cm (F6). The androgenic line F6 and the control showed the highest average fruit width (6.62 cm), while the lowest was recorded for F6 (5.84 cm). Consequently, the androgenic genotypes were characterized by significantly different ($p < 0.05$) fruit index as compared to the control. The highest mean fruit index was recorded for F6 (1.58) and the lowest for F5 (1.07). The control fruits were significantly ($p < 0.05$) heavier (98.61 g) as compared to the androgenic fruits (F5 – 88.84 g; F6 – 89.29 g; F7 – 79.41 g). The genotype F7 was considered with the lightest fruits as compared to Feherozon genotypes. Regarding the trait number of fruit locules, only F6 has shown significantly different number of fruit locules compared to Feherozon androgenic genotypes and the control Fk. The trait number of fruit locules showed significant variability ($p < 0.05$) only in the first experimental year, while the rest of studied fruit traits showed significant variability through each of the consequent experimental year (Table 3).

The mean fruit flesh (81.08%) of F6 androgenic genotype was significantly superior as compared to the other androgenic genotypes, where the lowest mean percentage was recorded for of control Fk. On contrary, the same genotype showed significantly the thinness pericarp wall of 0.45 cm. The mean percentage of dry matter of fresh fruit of all androgenic genotypes (F5 - 6.53%; F6 – 6.34%; F7 – 6.52) were significantly superior as compared to the control Feherozon (5.73%). The traits fruit flesh and percentage of fruit dry matter showed through each of the consequent experimental year significant variability only in the first experimental year. On the contrary, the pericarp thickness mean values varied significantly only in I and IV the experimental year (Table 4).

There are studies on fruit traits of androgenic pepper lines obtained from hybrids, result of crossing of different pepper species. The fruit traits were not compared to the parental genotypes, but to the fruits of the hybrid [19-21]. The results for morphological traits of fruits of androgenic plants of hybrid-crosses between *C. frutescens* L. and *C. chinense* Jacq. are in agreement with our results regarding to variability of traits and their stability in their consequent generations of testing [22]. The results of agromorphological traits of androgenic fruits from the variety Boogie F1 are in agreement with our results for the fruit length, width and weight [7]. Most of the androgenic lines originated from the variety Hebar were characterized with uniformity

and higher values of the studied fruit traits than initial variety, except for fruit width and fruit wall thickness [8]. Nowaczyk et al. have found that seventy-four percent of studied androgenic plants were different compared to the mother genotype in ripe fruit color, pericarp thickness and fruit taste. Other nine diploid androgenic plants showed differences from the mother genotypes in fruit weight, wall thickness and dry matter content [23].

However, still there is a dearth of information regarding research on morphological traits, including fruits of androgenic pepper genotypes although many authors reported production of haploid and dihaploid pepper plants via androgenesis [24-27]. One of the reasons for lack of data on evaluation of androgenic pepper plants in real cultivation conditions is due to low regeneration rate of androgenic plants [27-28]. Additionally, very often the androgenic pepper plants are evaluated through molecular biology tools, which on one hand gives quick results about the inheritance characteristics of the plants and their difference or similarity to the parental genotype [29]. On the other hand, the androgenic pepper plants are not tested in real cultivation conditions, thus, there is no information about their genotype expression under specific abiotic and biotic factors [30-31].

3.2 Assessment of Production Traits of Androgenic Genotypes

The results which characterize production trait of Kurtovska Kapia genotypes under this study are presented in Table 5. The average number of fruits per plant during the whole experimental period was 5.19 fruits (KKK), 6.22 fruits (KK1) and 4.23 fruits (KK2) but without statistically significant difference ($p < 0.05$). The trait yield per plant varied from 252.06 g (KK2) to 330.87 (KK1). Consequently, the highest total yield was recorded for androgenic genotype KK1 (2.07 kg/m²) and the lowest for KK2 (1.58 kg/m²). The average yield of the variety Kurtovska Kapia is 50 t/ha in open field production [15].

The results which characterize production trait of Feherozon genotypes under this study are presented in Table 6. The average number of fruits per plant during the whole experimental period varied from 4.54 fruits (Fk) to 5.25 (F6) without statistically significant difference ($p < 0.05$). The trait yield per plant varied from 329.07 g (F7) to 377.40 (F5). Moreover, the highest total yield was recorded for androgenic genotype F5 (2.36 kg/m²) and the lowest for the

control F7 (2.06 kg/m²). The average yield of the variety Feherozon under protected conditions is 6-8 kg/m² and 25-35 t/ha in open field conditions [15].

Table 3. Assessment of fruit traits length, width, index, weight and number of locules of androgenic lines F5, F6, F7 and the control Feherozon (Fk)

Experimental year	Genotype	Fruit length (cm)	Fruit width (cm)	Fruit index	Fruit weight (g)	Number of fruit locules
I	Fk	8.46b	6.29bc	1.35b	101.82a	3.40a
	F5	6.03c	6.60a	0.92c	79.8b	3.05ab
	F6	9.55a	5.35c	1.82a	66.75b	2.79b
	F7	5.81c	6.02b	0.97c	67.49b	3.06ab
II	Fk	6.99b	6.76a	1.04b	102.72a	3.70a
	F5	6.94b	6.78a	1.03b	101.52a	3.65a
	F6	9.53a	6.10b	1.56a	102.27a	3.40a
	F7	6.55b	6.32b	1.04b	86.72b	3.50a
III	Fk	8.98a	6.82ab	1.32ab	112.19a	3.30a
	F5	8.15a	7.13a	1.16bc	109.30a	3.20a
	F6	8.98a	6.59b	1.38a	102.06a	3.00a
	F7	6.83b	6.76ab	1.02c	100.27a	3.35a
IV	Fk	7.46b	6.37a	1.18b	79.30a	3.30a
	F5	6.84bc	6.04a	1.13bc	64.96b	3.30a
	F6	9.56a	6.02a	1.61a	83.05a	3.15a
	F7	6.17c	6.04a	1.03c	61.95b	3.45a
Average I-IV	Fk	8.14 ^{b,2}	6.62 ^{a,23}	1.21 ^{b,2}	98.61 ^{a,2}	3.46 ^{a,2}
Average I-IV	F5	6.81 ^{c,1}	6.62 ^{a,3}	1.07 ^{c,1}	88.84 ^{b,12}	3.24 ^{a,12}
Average I-IV	F6	9.40 ^{a,3}	5.84 ^{c,1}	1.58 ^{a,3}	89.29 ^{b,12}	3.00 ^{b,1}
Average I-IV	F7	6.27 ^{d,1}	6.29 ^{b,2}	1.03 ^{c,1}	79.41 ^{c,1}	3.25 ^{a,12}

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 4. Assessment of fruit traits fruit flesh, pericarp thickness and dry matter of androgenic lines F5, F6, F7 and the control Feherozon (Fk)

Experimental year	Genotype	Fruit flesh (%)	Pericarp thickness (cm)	Dry matter in fresh fruit (%)
I	Fk	89.12a	0.57a	7.00b
	F5	87.96a	0.48b	7.86a
	F6	90.66a	0.42b	8.11a
	F7	88.10a	0.46b	8.32a
II	Fk	79.89c	0.45a	5.65b
	F5	81.83c	0.41a	5.93ab
	F6	97.76a	0.42a	5.95ab
	F7	84.60b	0.46a	6.25a
III	Fk	64.73b	0.42a	5.0b
	F5	67.90ab	0.44a	5.55ab
	F6	74.57a	0.44a	5.55ab
	F7	70.35ab	0.44a	5.88a
IV	Fk	67.90b	0.47b	5.90b
	F5	64.29c	0.49ab	6.85a
	F6	71.82a	0.47b	5.85b
	F7	64.86bc	0.47b	5.83b
Average I-IV	Fk	73.45 ^{b,1}	0.48 ^{a,2}	5.73 ^{b,1}
Average I-IV	F5	75.33 ^{b,1}	0.45 ^{a,12}	6.53 ^{a,2}
Average I-IV	F6	81.08 ^{a,2}	0.42 ^{b,1}	6.34 ^{a,2}
Average I-IV	F7	76.70 ^{b,12}	0.45 ^{a,12}	6.52 ^{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 5. Productivity characteristics of androgenic genotypes KK1 and KK2 and the control Kurtovska Kapia (KKk)

Experimental year	Genotype	Number of fruits per plant	Yield per plant (g)	Total yield (kg/m ²)
–	KKk	5.25a	223.50a	1.40a
	KK1	5.03a	277.37a	1.73a
	KK2	3.55a	229.75a	1.44a
=	KKk	3.60a	165.05a	1.03a
	KK1	5.56a	301.50a	1.88a
	KK2	3.55a	212.13a	1.33a
≡	KKk	7.75a	444.50a	2.78a
	KK1	8.35a	481.50a	3.07a
	KK2	5.38a	334.00a	2.09a
≥	KKk	4.10a	223.25a	1.40a
	KK1	4.22a	227.33a	1.42a
	KK2	4.50a	225.83a	1.41a
Average I-IV	KKk	5.19 ^{a,1}	264.08 ^{a,1}	1.65 ^{a,1}
Average I-IV	KK1	6.22 ^{a,1}	330.87 ^{a,1}	2.07 ^{a,1}
Average I-IV	KK2	4.23 ^{a,1}	252.06 ^{a,1}	1.58 ^{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 $P<0.01$

Table 6. Productivity characteristics of androgenic genotypes F5, F6, F7 and the control Feherozon (Fk)

Experimental year	Genotype	Number of fruits per plant	Yield per plant (g)	Total yield (kg/m ²)
–	Fk	5.65a	333.50a	2.08a
	F5	6.33a	391.50a	2.45a
	F6	7.03a	371.00a	2.32a
	F7	6.38a	323.50a	2.02a
=	Fk	4.20a	354.5a	2.22a
	F5	4.95a	397.75a	2.49a
	F6	4.88a	362.88a	2.27a
	F7	5.05a	349.50a	2.19a
≡	Fk	4.85a	425.50a	2.83a
	F5	5.48a	472.75a	2.96a
	F6	5.33a	467.75a	2.92a
	F7	4.95a	431.25	2.70a
≥	Fk	3.45a	256.60a	1.60a
	F5	3.38a	203.00a	1.27a
	F6	3.28a	229.25a	1.43a
	F7	2.65a	173.00a	1.08a
Average I-IV	Fk	4.54 ^{a,1}	349.28 ^{a,1}	2.18 ^{a,1}
Average I-IV	F5	5.15 ^{a,1}	377.40 ^{a,1}	2.36 ^{a,1}
Average I-IV	F6	5.25 ^{a,1}	366.29 ^{a,1}	2.29 ^{a,1}
Average I-IV	F7	4.91 ^{a,1}	329.07 ^{a,1}	2.06 ^{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 $P<0.01$

Based on field observation during the whole experimental period, the small number of fruits per plant and low total yield of studied genotypes was due to late sowing and high air temperatures during flowering and fertilization which resulted in high flower abortion percentage and low fertilization rate [32-33]. This observation is in

agreement with the results of the yield of pepper androgenic lines studied in open field [8]. On the other hand, one of the aims of the experiment was to collect as many seeds as possible in each consequent generation for future research purposes. Therefore, most of the fruits were harvested in fully ripen stage which directly affect

the fruit number per plant and the total yield of each androgenic genotype. These findings are in agreement with Gvozdenović (2009) that when pepper fruit is utilized in fully mature stage the total yield is mostly lower compared to the harvests in unmaturing or intermediate stage of pepper fruit [34].

Shrestha et al. for the androgenic lines have found high positive and negative variability as compared to the parental genotype which is in disagreement with our results [7]. Other researchers found that all anther-derived lines were with higher values of total yield compared to the initial variety Hebar [8]. However, the fruit number per plant of tested androgenic genotypes is in agreement with the results for the same trait for androgenic lines AP1, AP2, AC9 и AC14 reported by Olszewska et al. [21].

3.3 Classification of Androgenic Genotypes According to Fruit and Productivity Traits

3.3.1 Androgenic genotype KK1

Androgenic genotype KK1 was developed from the Macedonian variety Kurtovska Kapia by the method of androgenesis. According to the fruit weight (65.05 g) and the fruit index higher than 2, the fruit is classified as long and triangular large-size pepper. The fruit flesh of 77.86% and the pericarp thickness 0.37 cm characterize this fruit as medium-fleshy. These fruits are suitable for fresh consumption, roasted and processed in form of different dips. This genotype is characterized by 6.22 fruits per plant averagely weighing 330.87 g and total yield of 2.07 kg/m². According to production traits, in the given agroecological and cultivation conditions, it is a medium-yield pepper genotype.



Fig. 1. Fruits of androgenic genotype KK1

3.3.2 Androgenic genotype KK2

Androgenic genotype KK2 was developed from the Macedonian variety Kurtovska Kapia by the method of androgenesis. The fruit is characterized with weight of 71.20 g, 6.95% dry matter, 79.06% fruit flesh and 0.36 cm pericarp thickness. KK2 fruit is classified as long, triangular large-size and medium-fleshy pepper. The KK2 total yield gained in this research is 1.58 kg/m². One plant is averagely loaded with 4.23 fruits weighing 252.06 g. According to production traits, in the given agroecological and cultivation conditions, it is a low-yield pepper genotype. Likewise KK1, this genotype is suitable for utilization as fresh, roasted and processed in form of different dips.



Fig. 2. Fruits of androgenic genotype KK2

3.3.3 Androgenic genotype F5

Androgenic genotype F5 was developed from the Hungarian variety Feherozon by the method of androgenesis. According to the fruit weight (88.84 g) and the fruit index higher than 1, the genotype is classified as bell-shaped, large-size peppers. The fruit flesh of 75.33% and the pericarp thickness 0.45 cm characterize this fruit as very thin. This genotype is characterized by 5.15 fruits per plant averagely weighing 377.40 g and total yield of 2.36 kg/m². According to production traits, in the given agroecological and cultivation conditions, it is a medium-yield pepper genotype. The fruits are suitable for fresh consumption, cooking and pickling.

3.3.4 Androgenic genotype F6

Androgenic genotype F6 was developed from the Hungarian variety Feherozon by the method of androgenesis. The fruit weight (89.29 g) and the fruit index 1.58 classify the genotype as blocky-elongated, large-size peppers. The fruit flesh of

81.08% and the pericarp thickness 0.42 cm characterize this fruit as very thin and make it suitable for fresh consumption, cooking and pickling. This genotype is characterized by 5.25 fruits per plant averagely weighing 366.29 g and total yield of 2.29 kg/m². According to production traits, in the given agroecological and cultivation conditions, it is a medium-yield pepper genotype.



Fig. 3. Fruits of androgenic genotype F5



Fig. 4. Fruits of androgenic genotype F6

3.3.5 Androgenic genotype F7

Androgenic genotype F7 was developed from the Hungarian variety Feherozon by the method of androgenesis. According to the fruit weight (79.41 g) and the fruit index 1.03, the genotype is classified as bell-shaped large-size peppers. The fruit flesh of 76.70% and the pericarp thickness 0.45 cm characterize this fruit as thin pepper. This genotype is characterized by 4.91 fruits per plant averagely weighing 329.07 g and total yield of 2.06 kg/m². According to production traits, in the given agroecological and cultivation conditions, it is a medium-yield pepper genotype. The genotype is suitable for utilization as fresh, for cooking and pickling.



Fig. 5. Fruits of androgenic genotype F7

4. CONCLUSION

The assessment of fruit and production traits of androgenic genotypes under study offers valuable material for future breeding. Androgenic genotypes KK1 and KK2 differ from the control variety for four fruit traits. Androgenic genotypes F5, F6 and F7 differ from the control variety for six fruit traits. There was no variability of the productivity traits between studied androgenic genotypes and their parental genotypes. From the assessment of androgenic genotypes as compared to the control genotypes Kurtovska Kapia and Feherozon, all five androgenic genotypes, are recommended for future variety development of sweet pepper varieties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bosland PW, Votava EJ. Peppers: Vegetable and spice capsicums. New York: CABI Publishing; 2000.
2. FAOSTAT. Available:<https://faostat.fao.org>
3. Crosby K, Pike L, Jifon J, Yoo K. Breeding vegetables for optimum levels of phytochemicals. Proceedings of FAV 2005, Quebec City, Canada; 2005.
4. Koleva Gudeva L, Trajkova F, Zlatkovski V. Biotechnology and biodiversity: Aspects of improvement of genetic resources of agriculture crops. Yearbook of Institute of Faculty of Agriculture, Goce Delcev University – Stip. 2008;7:57–66.
5. Simaroo Segui JM. Androgenesis in Solanaceae. In: Germanà MA, Lambardi M. editors. *In vitro* embryogenesis in higher

- plants, methods in molecular biology. New York: Springer Science+Business Media. 2016;1359.
6. Irikova T, Grozeva S, Rodeva V. Anther culture in pepper (*Capsicum annuum* L.) *in vitro*. Acta Physiologiae Plant. 2011;33: 1559-1570.
 7. Shrestha SL, Luitel BP, Kang WH. Agromorphological characterization of anther derived plants in sweet pepper (*Capsicum annuum* L. cv. Boogie). Horticulture, Environment, and Biotechnology. 2011; 52(2):196-203.
 8. Todorova V, Grozeva S, Rodeva V, Masheva S. Breeding evaluation of pepper lines obtained by *in vitro* anther culture. Genetika. 2013;45(2):601-10.
 9. Kisiata A, Olszewska D, Niklas-Nowak A, Nowaczyk P. Biometrical characteristics of r2 generation of anther-derived pepper (*Capsicum* spp.) plants. Acta Agrobotanica. 2011;64(3):53-58.
 10. Trajkova F, Koleva Gudeva L. Evaluation and agronomic potential of androgenic pepper genotypes derived from Piran (*Capsicum annuum* L. cv. Piran). Journal of Experimental Agriculture International. 2017;16(4):1-2.
 11. Dumas de Valux R, Chambonnet D, Pochard E. *In vitro* culture of pepper (*Capsicum annuum* L.) Anthers: High rate plant production from different genotypes by +35°C treatments. Agronomie. 1981; 1(10):859-864.
 12. Koleva Gudeva L, Trajkova F, Dimeska G, Spasenoski M. Androgenesis efficiency in anther culture of pepper (*C. annuum* L.). Acta Horticulturae. (ISHS). 2009;830:183-190.
 13. Koleva Gudeva L, Trajkova F. Seed production from pepper obtained in *in vitro* anther culture. Yearbook of Institute of Southern Crops – Strumica 2004/2005. 2005;4(1):85-93.
 14. Koleva Gudeva L, Trajkova F. Application of androgenesis as method for improvement of agricultural crops diversity. III Congress of ecologists of Macedonia with international participation. Book of proceedings. 2008;284-290.
 15. Trajkova F. Characterization and agronomic evaluation of some pepper lines (*Capsicum annuum* L.) obtained via androgenesis. Faculty of Agriculture, Ss. Cyril and Methodius University – Skopje. 2013;149.
 16. IPGRI, AVRDC and CATIE. Descriptors for *Capsicum* (*Capsicum* spp.). International Plant Genetic Resources Institute, Rome, Italy; the Asian Vegetable Research and Development Center, Taipei, and the Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica; 1995.
 17. Jankuloski D. Study of biological, morphological and qualitative traits of important populations of long peppers in Macedonia. PhD Thesis. Skopje: Faculty of Agriculture; 1983.
 18. Jankuloski D. Pepper and eggplant. Skopje: NIP BAS-TRADE; 1997.
 19. Olszewska D, Niklas-Nowak A, Nowaczyk P. Variation in the quantitative characters of androgenic pepper lines derived from hybrid *Capsicum frutescens* L. x *C. chinense* JACQ. Vegetable Crops Research Bulletin. 2010;73(1):5–11.
 20. Olszewska D, Niklas-Nowak A, Nowaczyk P. Variation in the quantitative characters of androgenic pepper lines derived from hybrid *Capsicum frutescens* L. x *C. chinense* JACQ. Vegetable Crops Research Bulletin. 2010;73(1):5–11.
 21. Olszewska D, Niklas-Nowak A, Nowaczyk P. The assessment of doubled haploid lines obtained in pepper (*Capsicum annuum* L.) anther culture. Folia Horticulturae. 2011;23(2):93-99.
 22. Kisiata A, Olszewska D, Niklas-Nowak A, Nowaczyk P. Biometrical characteristics of r2 generation of anther-derived pepper (*Capsicum* spp.) plants. Acta Agrobotanica. 2011;64(3):53-58.
 23. Nowaczyk L, Nowaczyk P, Olszewska D. Genetic analysis of anther culture-derived diploids of *Capsicum* spp. The Journal of Horticultural Science and Biotechnology. 2015;90(6):747-52.
 24. Mitykó J, Gemes Juhasz A. Improvement in the haploid technique routinely used for breeding sweet and spice peppers in Hungary. Acta Agronomica Hungarica. 2006;54:203-209.
 25. Rodeva V, Grozeva S, Todorova V. *In vitro* answer of Bulgarian pepper (*Capsicum annuum* L.). Genetika. 2006;38(2):129-136.
 26. Supena EDJ, Suharsono S, Jacobsen E, Custers JB Successful development of a shed-microspore culture protocol for doubled haploid production in Indonesian

- hot pepper (*Capsicum annuum* L.). Plant cell reports. 2006;25(1):1-10.
27. Pauk J, Lantos C, Somogyi G, Vági P, Ábrahám Táborosi Z, Gémes Juhász A, Tímár Z. Tradition, quality and biotechnology in Hungarian spice pepper (*Capsicum annuum* L.) breeding. Acta Agronomica Hungarica. 2010;58(3):259-266.
 28. Mitykó J, Fari M. Problems and results of doubled haploid plant production in pepper (*Capsicum annuum* L.) via anther and microspore culture. Acta Horticulturae. 1997;447:281-287.
 29. Mitykó J, Gémes Juhász A. Improvement in the haploid technique routinely used for breeding sweet and spice peppers in Hungary. Acta Agronomica Hungarica. 2006;54:203-209.
 30. Irikova TP, Kintzios S, Grozeva S, Rodeva V. Pepper (*Capsicum annuum* L.) anther culture: Fundamental research and practical applications. Turkish Journal of Biology. 2016;40(4):719-26.
 31. Lefebvre V, Palloix A, Caranta C, Pochard E. Construction of an intraspecific integrated linkage map of pepper using molecular markers and doubled-haploid progenies. Genome. 1995;38(1):112-121.
 32. Marcelis LFM, Heuvelink E, Hofman-Eijer LB, Den Bakker J, Xue LB. Flower and fruit abortion in sweet pepper in relation to source and sink strength. Journal of Experimental Botany. 2004;55(406):2261-2268.
 33. Erickson AN, Markhart, AH. Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. Plant, Cell & Environment. 2002;25(1):123-130.
 34. Gvozdenović Đ. Testing pepper varieties intended for production of vegetable caviar. Contemporary Agriculture. 2009; 58(1-2):12-18.

© 2017 Trajkova and Gudeva; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://sciencedomain.org/review-history/21839>