https://doi.org/10.61308/AOHQ9376

Comparative evaluation of melon plants obtained via somatic embryogenesis

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Citation: Velkov, N., & Ivanova, Zh. (2025). Comparative evaluation of melon plants obtained via somatic embryogenesis. *Bulgarian Journal of Crop Science*, *62*(3) 53-60

Abstract: A comparative evaluation of the progeny of melon line VK/1-5-5 obtained by somatic embryogenesis was carried out in "Maritsa" Vegetable Crops Research Institute – Plovdiv during the period 2022-2023. The aim of our study was to use the phenomenon of somaclonal variation to obtain new lines with improved qualities. A selection was made and parameters of the plant and fruits were reported according to twenty characterictics: days to ripening, vegetation period, fruit mass, fruit length, fruit diameter, flesh thickness, total soluable solids, flowering type, fruit ribbing, fruit pattern of cork formation, predominant fruit skin colour, fruit surface, fruit corking/ netting intensity, fruit shape in longitudinal section, main colour of flesh, aroma, flesh texture, taste, flesh flavor and flesh moisture. The seed progeny of the regenerated plants was compared among themselves and with line VK/1-5-5, which was used as a control. The results show a significant variation in the values of the traits in individual progeny. According to the indicator of days to fruit ripening, the shortest period was reported for E 70-1/1-4-asf (30 days), which was 7 days earlier than the control. The vegetation period is the shortest for the same genotype – 91 days. The average fruit weight was highest in E 70-1/2-4-8-8 (3.905 kg), twice that of the control. Other progeny with greater mass than the control were E 70-1/2-4-8-3 (2.529) and E 70-1/2-4-6-sf (2.817 kg). In the same genotypes, the highest values were obtained in terms of fruit length, fruit width and fruit thickness. The highest content of dry matter was obtained at E 70/1-5-20 - 12.1%. The investigated progenies of regenerants are characterized by monoecious and andromonoecious type of flowering and male sterility. The obtained changes in progeny are a valuable source of variability in the melon breeding program.

Kewords: Cucumis melo; in vitro; somaclonal variation; progeny

INRODUCTION

Melons are one of the important and traditional vegetable crops grown in Bulgaria. Their importance is determined by the taste and dietary qualities of the fruits, which are consumed fresh and processed (juices and smoothies) (Ivanova et al., 2013; Kumar et al., 2022). Increasing genetic diversity and obtaining plant genotypes with improved qualities is a major task in any breeding program. One of the ways to achieve genetic diversity is by using *in vitro* methods that are based on somatic embryogenesis or gametogenesis. Gametoclnal variation occurs *among the plants regenerated from gametic cultures*, and somaclonal variation arise in plants regenerated from somatic cell and tissue cultures. Most scientific studies claim that both methods are suitable for achieving wide variability (Skirvin et al., 1994; Pawełkowicz et al., 2021; Duta-Cornescu et al., 2023). In *Cucurbitaceae* crops gynogenesis is mainly applied, by introducing pistils or embryos obtained by irradiated pollen: cucumber (Deng et al., 2020; Baktemur et al., 2022); melon (Koli and Murthy, 2013; Nitwatthanakul et al., 2018), watermelon (Zhu et al., 2019). An increase in ge-

netic diversity through somatic embryogenesis is significant because DNA at early stages of development in somatic embryogenesis contains lower levels of methylation than at later stages (Sahijram et al., 2003).

One way to detect and characterize somaclonal variants is based on differences in morphological traits (Pérez et al., 2011; Nhut et al., 2013). In the case of the melon, important characteristics are fruit weight, length, width, thick of the fleshy part, color of the skin and flesh, presence of lobes, reticulation, dry matter content, taste, aroma, texture, etc. The type of flowering is important for obtaining hybrid varieties and especially the presence of male sterility or gynoecious type of flowering.

The factors responsible for the observed variability are due to the genotype, the composition of the culture medium, the type of explant, the condition of the donor plant, direct or indirect embryogenesis (Mohiuddin et al., 2021; Nunez-Palenius et al., 2008). In most studies scientists investigated changes in regenerated plants in result of direct or indirect embryogenesis. There are not enough investigations of the seed generations of the selected regenerants to establish in more detail the observed variability compared to the initial form.

In our previous studies, regenerated plants were obtained from somatic embryogenesis of the VK/1-5-5 line (Ivanova, 2021), which were seed propagated and a selection was made up to the third generation. The aim of our research is to study obtained seed progeny from the VK/1-5-5 line for important morphological characters of the fruits and plants, which information will contribute to their effective introduction into breeding programs.

MATERIAL AND METHODS

Plant material

The study was carried out at the „Maritsa“ Vegetable Crops Research Institute, Plovdiv, Bulgaria, during 2022-2023. Twelve seed progeny of melon line VK/1-5-5 were studied, which were obtained by somatic embryogenesis from our previous experiments (Ivanova, 2021). VK/1-5-5 line was used as a control to compare the studied traits. Melon line VK/1-5-5 belongs to var. cantalupensis which is described with monoecious type of flowering, male sterility (ms-4), fruits possess elliptical shape, yellow ground colour of skin, orange colour of the flesh.

Plants and fruits were phenotyped for seven quantitative traits:

- days to ripening (DR), The indicator is reported in the number of days from the date of flowering of the female (hermaphrodite) flower to the date of harvesting the fruit;
- vegetation period (VP) - days from plant germination to fruit harvesting;
- fruit mass (FM) (kg);
- fruit length (FL) (cm);
- fruit width (diameter) (FW) (cm);
- flesh thickness (cm) (FT);
- total soluble solids (TSS) (°Brix) were determined refractometrically KERN ORA 32 BA/BB).

Thirteen fruit traits were characterized according to UPOV (Table 2).

Terms of the experiment

The seeds were sown on 15 March in perlite substrate. Pricking were done on 22 March in 0.5 L pots. Plants were transplanted on 20 April. The plants were grown in greenhouse conditions according to technological requirements. A double-row system was used; the scheme of transplanting was 240 cm between the centers of each pair of rows, 80 cm between the two rows within a pair, 45 cm between plants in the rows. Plant density was 1.4 plants/m^2. Plants were grown vertically in the greenhouse until plants reached the support wires (200 cm). Plants were irrigated, fertilized and protected from pathogens and pests according to standard horticultural practices. Harvesting was performed from 20 – 26 June to 20 – 26 July.

Trial design

The trial was performed by a complete randomized block design in three replications, 0.72

m^2 nutrient plot per plant, ten plants per replication. Twelve melon genotypes were observed and line VK/1-5-5 were used as a control variant.

Statistical analysis

All data were statistically analyzed using the SPSS 19 software (SPSS Inc., Chicago, USA). Duncan's multiple range test was performed at $P \leq 0.05$ on each of the significant variables measured. Cluster analysis by average linkage (between groups) was conducted to identify similarities/dissimilarities between the studied variants and Principal Component Analysis.

RESULTS AND DISCUSSION

The results of the comparative test of twelve seed progeny and line VK/1-5-5 show significant variation in the values of the six quantitative traits studied (Table 1). The variation expressed by the coefficient of variation was highest in average fruit mass (48.37%), followed by fruit length (29.38%), dry matter content (20.82%) and thickness of meat (18.46%). For the other traits, the variation is between 6.68% and 13.92%. Duncan's test data analysis indicated that the differences obtained from the control variant VK/1-5-5 and seed progeny were proved in both higher and lower values for the six traits. Melon fruits from genotype E 70-1/1-4-asf ripen faster (30 days) than the control VK/ 1-5-5 (37 days), while the slower ripening is observed in E 70/1-5-2-2 (47 days). Fruit ripening in melons usually takes 30 - 70 days after flowering, such as control variant VK/1-5-5 belongs to the group of early ripening varieties. The longest vegetation period was observed in line VK/1-5-5 (115 days), and the shortest period was in genotype E 70-1/1-4-asf (91 days). In general, line VK/1-5-5 belongs to the

Table 1. Mean value of seven quantitative traits of 12 melon progeny obtained by *in vitro* methods

No	Genotype	Days to ripening		Vegetation period		Fruit mass (kg)		Fruit lenght (cm)		Fruit diameter (cm)		Flesh thickness (cm)		TSS % °Brix	
1	**E 70-1/1-4-asf**	30	g	91	h	1.219	de	12.4	e	14.2	cde	3.2	cd	7.3	cd
2	**E 70-1/1-4-bsf**	33	efg	94	fgh	1.138	de	12.7	e	13.0	def	2.8	de	6.9	cd
3	**E 70-1/1-4-csf**	31	fg	92	gh	1.341	de	12.9	e	14.4	cde	3.0	d	6.9	cd
4	**E 70-1/2-4-7-5**	35	d-g	96	e-h	0.714	e	14.4	de	10.7	f	2.1	e	6.3	d
5	**E 70-1/2-4-7-8**	39	bcd	100	cde	1.226	de	16.8	cd	12.2	ef	3.0	d	7.0	cd
6	**E 70-1/2-4-6-sf**	39	b-e	100	cde	2.817	b	19.9	bc	17.4	ab	4.3	a	9.7	abc
7	**E 70-1/2-4-8-5**	42	abc	103	bcd	2.347	bc	22.3	b	15.8	abc	3.5	a-d	9.7	abc
8	**E 70-1/2-4-8-3**	45	ab	106	bc	2.529	bc	23.2	b	16.6	abc	3.1	cd	9.3	a-d
9	**E 70-1/2-4-8-8**	42	abc	103	bcd	3.905	a	27.1	a	18.2	a	3.3	bcd	10.7	ab
10	**E 70/1-5-5-9**	39	cde	100	e-h	1.261	de	12.6	e	14.5	cde	3.5	a-d	9.3	a-d
11	**E 70/1-5-2-2**	47	a	108	b	1.376	de	12.4	e	14.8	b-e	3.8	abc	11.0	ab
12	**E 70/1-5-2-8**	37	c-f	98	d-g	1.894	cd	14.2	de	15.7	a-d	4.3	a	12.1	a
13	**VK/1-5-5 (C)**	37	c-f	115	a	1.856	cd	20.4	b	15.5	a-d	4.0	ab	8.5	bcd
	Mean	38		100		1.817		17.0		14.8		3.4		8.8	
	S±	5.1		6.7		0.879		5.0		2.1		0.6		1.8	
	CV%	13.40		6.68		48.37		29.38		13.92		18.46		20.82	
	Min	30		91		0.714		12.4		10.7		2.1		6.3	
	Max	47		115		3.905		27.1		18.2		4.3		12.1	

a,b,c – Duncan's Multiple Range Test, S± – Standard deviation; CV% - Coefficient of variation

group of medium-ripe varieties with a duration of the vegetation period of 80-110 days. In our experiments, earlier ripening forms were obtained, but nevertheless they fall into the group of medium-ripe melons. The duration of the growing season is determined by the period of the phenophases germination - flowering and fruit set - ripening. Therefore, selection can be carried out in the direction of earlier mature forms for one or both characteristics.

The average fruit mass is the main trait of productivity in melon. The results showed a significant variation in values from 0.714 kg to 3.905 kg. Fruit mass of line VK/1-5-5 (1.856 kg) is approximately averaged over the entire experiment. In most of the cases melon plants of current variety type (*cantalupensis*) produce one fruit per plant, therefore the higher fruit mass means higher productivity. The obtained genetic diversity shows that it is possible to increase the yield by increasing the weight and hence the size of the fruits. The size of the fruit, measured by the length, and thickness of the flesh, determines not only the productivity, but also the shape of the fruit. Respectively, the progeny with the highest fruit mass (E 70-1/2-4-6-sf, E 70-1/2-4-8-5, E 70-1/2-4-8-3 and E 70- 1/2-4-8-8) are also characterized by the largest fruit sizes. TSS content is important for fruit quality and is an indirect measure of sweetness and other organoleptic parameters. It is considered that the total soluble solids content recommended for melons sold in the markets should be higher or equal to 10 °Brix (Supapvanich et al., 2011). The tested genotypes E 70-1/2-4-8-8 E 70/1-5-2-2 E 70/1-5-2-8 are characterized by a higher TSS content, which can be considered very suitable for the market. The genotypes E 70-1/2-4-6-sf, E 70-1/2-4-8-5, E 70-1/2-4-8-3 and E 70/1-5-5-9 also have a high TSS content (9.3-9.7%), which makes them suitable for future parental components of F_1 hybrids.

The evaluation of the progeny according to 12 characteristics of the fruits and the flowering type of the plants also shows a significant variation from the initial genotype VK/1-5-5 (Table 2). Flowering type in melon is important for heterosis breeding, especially those possessing male sterility. Line VK/1-5-5 is distinguished by a *monoecious* flowering type and male sterility type ms-4 (Dogimont, 2011), which was the reason for choosing this line as a donor in our research. The obtained seed progeny are distinguished by monoecious and andromonoecious flowering type, and those marked with „sf“ also possess male sterility. It is interesting to note that the progeny E 70-1/1-4-asf, E 70-1/1-4-bsf and E 70-1/1-4-csf possess an andromonoecious flowering type with male sterility, as the sterility occurs in both male and hermaphrodite flowers. The characteristics of the fruits are important in determining the model of the variety. Fruit characteristics of melon line VK/1-5-5 are the most preferable by Bulgarian consumers. In our previous research, we established the current consumer requirements for some basic fruit characteristics (Ivanova et al., 2019). The presence of fruit ribbing varies from superficial (2) to intermediate (3). Different formations at the fruit surface indicate the maturity stage and also protect the fruit from mechanical injuries (Keren-Keiserman et al., 2004). Melon surfaces are most widely represented of dots and lines (3) to lines and nets (5). The predominant color of the fruit is important for the consumer, preferring those with an orange coloration (7), which was observed in nine genotypes, but also those with light yellow (2) and cream coloration (3) were found. The surface of the fruit in line VK/1-5-5 is heavily netted (9), with seven of the progeny showing lighty netted (8), and in one it is smooth (1). Fruit shape in longitudinal section varies considerably from ovate (1) to oblate (6). Main colour of flesh is determined by a combination of chlorophyll and carotenoid pigments, resulting in white, green, and orange colours (Burger et al., 2010). In the current experiment colour of flesh is presented in two categories, orange (7) and salmon (8). All genotypes have an aroma, but differ in other organoleptic parameters. The flesh texture is grainy (2), soft (3) and fibrous (4). Eight of the genotypes are distinguished with sweet taste (3), and five possessed intermediate taste (2). Flesh flavor, which is characterized by certain taste and aroma perceptions, is peach (1), forest fruit (3) and tropi-

Table 2. Mean values and frequency of 12 fruit traits and flowering type of 13 melon genotypes

Genotype	Flowering type	Fruit ribbing	Fruit pattern of cork formation	Predominant fruit skin colour	Fruit surface	Fruit corking/ netting intensity	Fruit shape in longitudinal section	Main colour of flesh	Aroma	Flesh texture	Taste	Flesh flavor	Flesh moisture
E 70-1/1-4-asf	2	2	3	3	8	2	6	8	2	2	3	3	2
E 70-1/1-4-bsf	2	2	3	7	1	2	6	7	2	2	2	3	2
E 70-1/1-4-csf	2	2	3	7	8	2	6	7	2	2	2	1	3
E 70-1/2-4-7-5	1	2	3	2	8	2	1	7	2	2	2	3	2
E 70-1/2-4-7-8	1	2	5	2	9	3	3	7	2	2	3	4	3
E 70-1/2-4-6-sf	1	3	5	7	9	3	3	7	2	3	3	3	3
E 70-1/2-4-8-5	1	3	5	2	9	3	2	7	2	4	3	3	3
E 70-1/2-4-8-3	1	3	5	7	8	3	3	8	2	2	2	4	3
E 70-1/2-4-8-8	1	3	5	7	9	3	3	7	2	2	3	4	2
E 70/1-5-5-9	2	2	3	7	8	2	6	7	2	4	2	1	2
E 70/1-5-2-2	2	3	4	7	8	3	6	7	2	2	3	4	2
E 70/1-5-2-8	2	3	4	7	8	2	6	7	2	2	3	4	2
VK/1-5-5 (C)	1	3	4	7	9	3	2	7	2	4	3	1	2
	Frequency												
1	7	0	0	0	1	0	1	0	0	0	0	3	0
2	6	6	0	3	0	6	2	0	13	9	5	0	8
3	0	7	5	1	0	7	2	0	-	1	8	5	5
4	0	0	3	0	0	0	4	0	-	3	0	5	-
5	-	-	5	0	0	-	0	0	-	0	-	0	-
6	-	-	0	0	0	-	6	1	-	-	-	0	-
7	-	-	-	9	0	-	0	10	-	-	-	0	-
8	-	-	-	0	7	-	0	2	-	-	-	0	-
9	-	-	-	0	5	-	-	-	-	-	-	0	-

Flowering type (1 monoecious, 2 andromonoecious); Fruit ribbing (1 absent, 2 superficial, 3 intermediate, 4 deep); Fruit pattern of cork formation (1 absent, 2 dots only, 3 dots and linear, 4 linear only, 5 linear and netted, 6 netted only).; Predominant fruit skin colour (1 white, 2 light yellow, 3 cream, 4 pale green, 5 green, 6 – dark green, 7 orange, 8 brown, 9 grey); Fruit surface (1 smooth, 2 grainy, 3 finely wrinkled, 4 deeply wrinkled, 5 shallowly wavy, 6 rare warts, 7 numerous warts, 8 lighty corked/ netted, 9 heavily corked/netted, 10 sutures); Fruit corking/ netting intensity (1 absent, 2 superficial, 3 intermediate, 4 pronounced); Fruit: shape in longitudinal section (1 ovate, 2 medium elliptic, 3 broad elliptic, 4 circular, 5 quadrangular, 6 oblate, 7 obovate, 8 elongated). Main colour of flesh (1 white, 2 yellow, 3 cream, 4 pale green, 5green, 6 pale orange, 7 orange, 8 salmon); Aroma (1 absent, 2 present); Flesh texture (1 firm, 2 grainy, 3 soft, 4 mealy, 5 fibrous); Taste (1 insipid, 2 intermediate, 3 sweet, 4 acetone); Flesh flavor (1peach, 2 ananas, 3 strawberry (forest fruit), 4 tropical fruit, 5 vanilla, 6 acetone, 7 pumpkin, 8 watermelon, 9 cucumber); Flesh moisture (1 Low, 2 Intermediate, 3 high).

cal fruit (4). Flesh moisture is intermediate (2) to high (3).

The progeny that are received have specific characteristics that largely differ from the initial form line VK/1-5-5. In order to summarize the obtained results, we performed cluster analysis and principal component analysis for the quantitative traits (Figure 1). The results of the cluster analysis show that the two distinct clusters were identified. The first cluster contains the genotypes E 70-1/1-4-asf, E 70-1/1-4-bsf, E 70-1/1-4-csf, E 70-1/2-4-7-5, E 70-1/2-4-7-8 and E 70/1-5-5-9, which are characterized by a short period of fruit ripening and vegetation period, with relatively large to medium-sized fruits and total soluble solids content up to 9%. The second cluster is divided into two subclusters. The first subcluster includes the genotypes E 70/1-5-2-2 and E 70/1-5-2-8, which are characterized by high TSS content, medium-sized fruits and flesh thickness. The second sub-cluster contains all the other genotypes, including the initial form VK/1-5-5, which are characterized by relatively large fruits, average length of the vegetation period and a TSS content of about 9%. In order to explain the grouping of the tested genotypes in clusters, was performed Principal Component Analysis (Table 3). The analysis of common components shows that a significant part of the variation is due to the first three components, which explain 91.46% of the total variation. The first component being with the greatest relative influence for the distribution of the genotypes explains 61.63% of the total variation. The correlations of the traits fruit weight, fruit length and fruit diameter are strong with the first component. The second principal component explains 15.86% of the total variation and is related to the traits flesh thickness and TSS. The effect of the third component is 13.96% and it correlates with the characters days to ripening and vegetation period. According to Biabani and Pakniyat (2008), traits from the same component are influenced by the same gene or closely related genes. In this case, traits with certain values lo-

Table 3. Total Variance Explained and Component Matrix using method of Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization

	Component		
Traits	**1**	**2**	**3**
Days to ripening	0.259	0.233	**0.859**
Vegetation period	0.189	0.218	**0.876**
Fruit weight	**0.917**	0.342	0.171
Fruit lenght	**0.906**	-0.029	0.378
Fruit diameter	**0.728**	0.637	0.131
Flesh thickness	0.096	**0.934**	0.168
TSS	0.260	**0.817**	0.374
% of Variance	61.63	15.86	13.96
Cumulative %	61.63	77.50	91.46

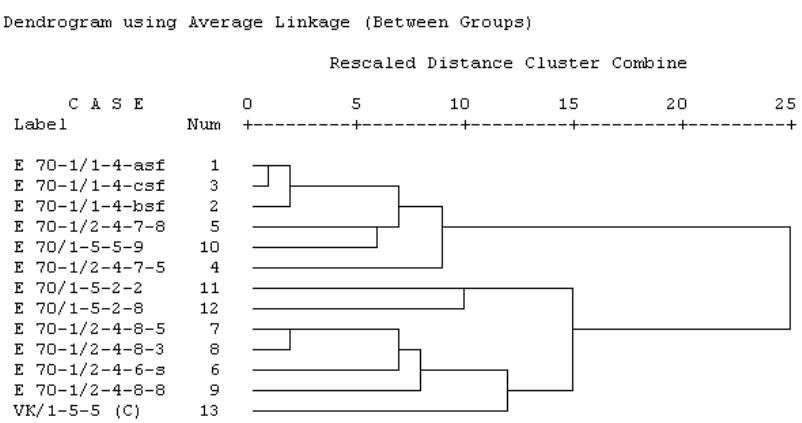

Figure 1. Hierarchical cluster analysis on the base of seven qualitative characters

cated in different components can be combined. Therefore, the traits fruit mass, TSS content and days to ripening can be combined in one genotype.

High variability in individuals received show the effectiveness of using somaclonal variation as a biological phenomenon. A number of studies indicate that *in vitro* methods has provided a new and alternative tool to breeders for obtaining genetic variability (Krishna et al., 2016; Pawełkowicz et al., 2021; Duta-Cornescu et al., 2023). In our experiment, we used third-generation of seed-propagated genotypes obtained from line VK/1-5-5, in which the changed characteristics are fixed and could be stably transfered to subsequent generations.

The obtained new genotypes are a valuable source in breeding process, which can continue with the testing of their combinatory ability and the development of new melon hybrid varieties.

CONCLUSION

Based on new forms of melons obtained through embryogenesis, twelve genotypes were selected and propagated by seeds. The tested seed progeny are characterized by high variability, which is due to somaclonal variation. The comparative evaluation shows that forms with significantly lower and higher values of fruit metric parameters were obtained. The variation in fruit quality indicators is also significant. A change in flowering type from monoecious to andromonoecious, with male sterility, was obtained. The obtaining progeny are a valuable source of diversity in melon breeding program. The genotypes E 70-1/2-4-6-sf, E 70-1/2-4-8-5, E 70-1/2-4-8-3 and E 70-1/2-4-8-8 are distinguished by the largest fruits, high TSS content and good external and organoleptic fruit characteristics.

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Received: December, 25, 2024; Approved: April, 06, 2025; Published: June, 2025