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GENETIC-BREEDING VALUE OF THE TOMATO FORMS CARRYING THE *β* (CAROTENE) AND *R* (YELLOW FLESH) GENES

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Abstract

One of the main problems of modern vegetable growing is the creation of varieties with complex valuable traits, adapted to growing conditions and economic efficiency. The forms containing the β (carotene) genes and r (yellow flesh), which play an important role in the diet of allergenic people, especially children, are of particular importance for improving red tomatoes. To demonstrate the variability of agronomic characters and to elucidate the value of tomato genotypes carrying β (carotene), r (yellow pulp) genes, a comparative assessment of tomato genotypes was made taking into account a set of useful traits. The evaluation of genotypes was carried out based on the most valuable biological parameters (period of vegetation, number of fruits and productivity per plant, fruit mass, thickness of pericarp, heat resistance). Genotypes combining characters of early ripeness with high productivity were identified in the tomato collection. Analysis of tomato genotypes for heat resistance made it possible to reveal highly resistant genotypes that are of interest as an initial material for breeding.

Key words: *tomato, breeding, early ripeness, resistance, heat.*

INTRODUCTION

Drought and high temperatures in last decades of growing season became factors which significantly limited growth, reproduction and productivity of crop plants including tomatoes in the Republic of Moldova (Mihnea, 2016; Mihnea, 2018).

The optimum temperature for growing tomatoes is between 25-30^{0}C during the day and 20^{0}C at night (Camejo et al., 2005; Ribeiro et al., 2008; Carvalho et al., 2011). Temperatures above 35^{0}C affect seed germination, vegetative growth, flowering, fruit binding and ripening (Thomas & Prasad, 2003; Wahid et al., 2007). High temperatures can cause significant losses in fruit productivity and quality (Stevens & Rudich, 1978; Firon et al., 2006; Wahid et al., 2007; Pervez et al., 2009; Nahar & Ullah, 2012).

The yield and quality of tomato fruits depend both on the optimal conditions for plant growth and on the use of varieties with proper genetic characteristics. This indicator is a decisive factor for innovative progress in agriculture and ensures high quality and quantity of production including organoleptic properties (Seymour et al., 2002; Ercolano et al., 2008; Carli et al., 2011; Mihnea et al., 2016). The knowledge about the variability of characters which is determined not only by genotype but also by environmental factors has the crucial importance. The degree of variability of characters indicates the peculiarities of the reaction norm of the genotype under various environmental conditions (Haydar et al., 2007; Mohamed, Ali & Mohamed, 2012). The information about the variation of the character determined by the variability of the genotype indicates the possibility of changing the parameter in the necessary direction at this stage of selection. Establishing the specificity of variability and heritability of characters gives the breeder the opportunity to optimize the breeding program (Fasoylas, 1973).

For the efficient use of the tomato gene pool by both researchers and producers, it is necessary to create a special collection of tomatoes with identified genes. Within each collection, it is necessary to select genotypes with the most valuable characters for the selective improvement of the species genes.

Diversification of tomato germplasm, supplementation of new genotypes carrying *β* (carotene) and *r* (yellow flesh) genes in red tomatoes play an important role for the diet of persons with an allergy, especially children.

The aim of our study was to evaluate the collection of tomatoes carrying the β (carotene) and *r* (yellow pulp) genes according to a set of useful traits (early ripeness, yield, fruit size and quality, resistance to high temperatures) and select the most valuable forms for further breeding.

MATERIALS AND METHODS

As research material, 22 genotypes of tomatoes were used, 15 of all analyzed varieties (Rufina, Charovnita, Rosinca, Viking, Gold Nugget, Golden Jubilee, Timisorean landrace, MilOrang, Luci, Alex, Flacara, Chihlimbar, Hurma, Breeding Line, Mia) have the β (carotene) gene and 7 varieties (Dolgonosic, Buyan yellow, Oranjevie sosulki, Moldavian landrace, L 10B, Vrojainii, De-barao yellow) carry the *r* (yellow flesh) gene. The experiments were carried out in laboratory and field conditions, in the experimental field of the Institute of Genetics, Physiology and Plant Protection (Chisinau, Moldova). Resistance to high temperatures was studied under laboratory conditions. The analysis of the variability of the resistance was made based on the length of the embryonic root, stem and seedling.

The following regimes were used to analyze the influence of high temperatures on the seedling: A = 38^0C; B = 40^0C; C = 42^0C. The exposure was 6 hours. Thus, a differentiated background was created for the selection of forms resistant to high temperatures. Methodological recommendations were used to assess resistance of tomato genotypes to high temperatures (Ivachin, 1979) on the base of the capacity of embryonic roots to grow after maintaining them at high temperatures within 6 hours.

Cluster analysis was made by creating dendrograms on the base of agglomerative-iterative algorithm (Ward method) and the k-means method (Savary, 2010). Four clusters were programmed within the k-means method.

Tomatoes were grown by seedling cultivation in three repetitions according to the standard method (Ersova, 1978). Under field conditions, the morphological description was made according to the UPOV descriptor (2011). Seedlings were planted in the field in the third decade of May. The data obtained were statistically processed using the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

As a result of evaluating the forms of tomatoes by precocity, a rather high variability of the growing season and interphase periods was revealed depending on both the genotype and climatic conditions (Figure 1). Variability of the interphase period from mass appearance of seedlings to the beginning of flowering was within the ranges of 63-78 days. It was shown that climatic conditions significantly influenced the first interphase period. This is due to low temperatures and cold nights in the spring of 2020, which led to a later flowering of some varieties. The varieties Golden Jubilee, Timisoranean local population, Chihlimbar, Mia, De-barao yellow showed a later flowering. Ranges of the period from flowering to fruit ripening were within 39-64 days. A shorter period was registered in the varieties Dolgonosic, Mia, Gold Nugget, Luci.

The tested genotypes formed 4 groups of precocity: early - 106-110 days (5, 9, 10, 12 16), medium early - 111-115 days (1, 3, 4, 8, 13, 18, 19, 21, 22), late - 116-120 days (14, 15, 20), very late > 120 days (2, 6, 7, 11, 17) (Figure 1).

As a result of the evaluating the forms on the base of characteristics of the fruit, it was found a rather high variability (Table 1). In studied groups, the highest coefficient of variation has the fruit mass, the average being 31.2%, and the thickness of the pericarp - 21.0%. The data showed a wide range of variability in fruit length and width, mesocarp thickness and the number of locules. Average levels of parameters were of 12.8%, 14, 6%, 18.1% and 18,9 %. Thus, the medium variability of these analyzed characters in the studied groups was demonstrated.

When constructing dendograms, genotypes were divided into 2 clearly separated branches based on the classification of the following indicators: the mass of the fruit, the length and width of the fruit, the thickness of the pericarp, the thickness of the mesocarp, the number of locules. The highest similarity was recorded for varieties 3, 21, 11, 22, 19 and 1, 6, 8, 12, 13 (Figure 2).

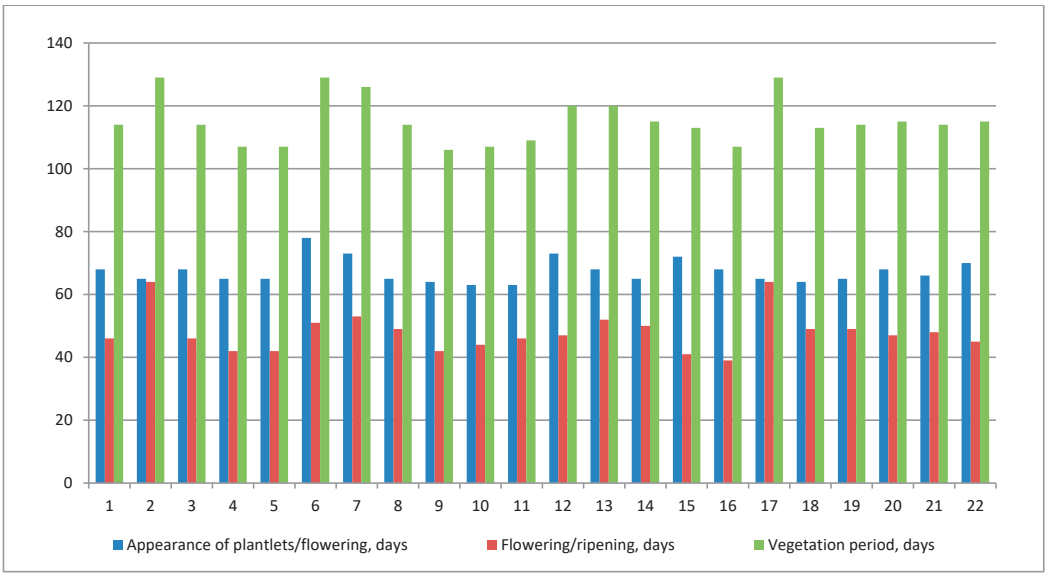


Figure 1. Phenotypic variability of interphase periods in tomato
1 – Rufina, 2 – Charovnita, 3 – Rosinca, 4 – Viking, 5 – Gold Nugget, 6 – Golden Jubilee, 7 – Timisorean landrace, 8 – MilOrang, 9 – Luci, 10 – Alex, 11 – Flacara, 12 – Chihlimbar, 13 – Hurma, 14 – Breeding Line, 15 – Mia, 16 – Dolgonosic, 17 – Buyan yellow, 18 – Oranjevie sosulki, 19 – Moldavian landrace, 20 – L 10B (Buzau), 21 – Vrojainii, 22 – De-barao yellow

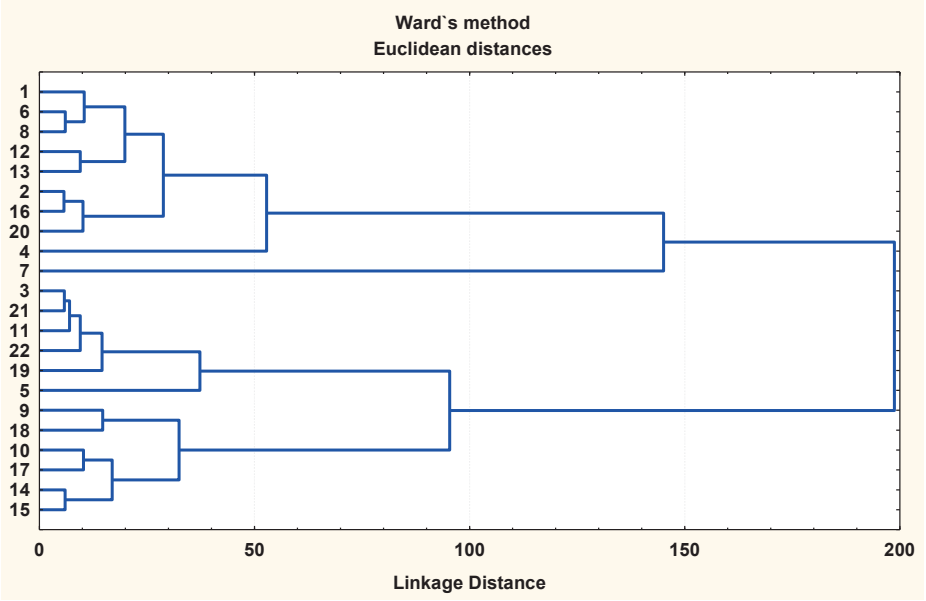


Figure 2. Cluster analysis of tomato varieties based on some fruit characteristics.
1 – Rufina, 2 – Charovnita, 3 – Rosinca, 4 – Viking, 5 – Gold Nugget, 6 – Golden Jubilee, 7 – Timisorean landrace, 8 – MilOrang, 9 – Luci, 10 – Alex, 11 – Flacara, 12 – Chihlimbar, 13 – Hurma, 14 – Breeding Line, 15 – Mia, 16 – Dolgonosic, 17 – Buyan yellow, 18 – Oranjevie sosulki, 19 – Moldavian landrace, 20 – L 10B (Buzau), 21 – Vrojainii, 22 – De-barao yellow

Table 1. Phenotypic variability of some fruit characteristics in tomato varieties

No.	Fruit mass, g		Fruit length, mm		Fruit diameter, mm		Pericarp thickness, mm		Mesocarp thickness, mm		Number of locules	
	$x \pm m_x$	V,%	$x \pm m_x$	V,%	$x \pm m_x$	V,%	$x \pm m_x$	V,%	$x \pm m_x$	V,%	$x \pm m_x$	V,%
1	65.2±3.37	23.2	43.8±0.95	9.8	50.8±1.28	11.3	6.6±0.31	21.2	36.4±1.20	14.8	3.9±0.22	25.6
2	54.6±3.28	26.9	48.3±2.95	27.3	45.3±1.47	14.6	5.0±0.15	12.8	30.0±1.30	19.4	2.9±0.13	20.7
3	36.0±2.29	28.3	35.2±0.86	10.9	39.2±0.86	8.5	3.6±0.20	25.0	26.4±0.65	11.1	3.1±0.15	22.6
4	87.4±8.0	39.9	41.8±0.88	9.1	60.3±2.29	16.6	3.5±0.19	24.0	46.1±1.85	17.6	6.6±0.21	13.6
5	14.4±0.94	29.2	29.1±0.62	9.6	27.8±0.64	10.4	3.2±0.15	21.9	15.2±0.77	22.7	2.2±0.09	18.7
6	56.3±10.39	52.2	37.8±0.94	7.1	48.4±4.43	26.0	3.9±0.30	21.3	35.5±2.74	22.0	5.3±0.25	13.2
7	146.5±7.18	17.7	50.3±2.87	20.6	63.5±3.93	22.4	6.4±0.30	16.1	49.5±3.10	22.7	5.5±0.40	27.3
8	58.7±3.71	28.2	42.9±1.14	11.9	49.0±1.75	16.0	4.7±0.15	15.0	35.1±0.71	9.11	3.6±0.11	13.9
9	40.4±1.81	22.3	66.7±1.36	9.1	33.6±0.61	8.1	5.4±0.21	17.4	19.4±0.70	16.3	3.0±0.09	13.3
10	48.2±2.05	19.1	49.5±0.97	8.7	42.9±0.91	9.6	3.8±0.19	21.9	24.1±1.92	35.7	2.9±0.14	22.1
11	33.7±2.28	30.3	39.3±0.93	10.7	38.1±0.90	10.5	4.7±0.19	19.1	23.2±0.92	17.8	2.7±0.11	18.5
12	55.1±4.52	29.6	49.8±3.20	23.1	51.6±3.23	22.7	5.2±0.34	23.6	41.3±2.32	20.3	4.4±0.18	15.9
13	58.1±6.84	52.5	45.4±2.03	4.6	49.0±1.88	17.1	3.9±0.20	23.3	48.3±1.75	15.8	6.6±0.41	18.2
14	38.9±3.42	39.3	52.3±1.65	14.1	35.8±1.18	14.8	7.2±0.44	27.8	21.8±0.82	16.5	2.3±0.11	21.7
15	35.9±1.17	14.8	56.1±1.87	15.0	33.5±0.55	7.5	5.7±0.24	19.3	19.7±0.69	15.8	2.4±0.11	20.8
16	57.4±2.23	17.4	53.2±1.00	8.5	44.8±0.51	5.1	5.6±0.21	16.8	29.7±1.34	20.2	2.5±0.11	20.4
17	46.2±6.23	48.7	54.1±3.37	22.4	34.5±3.02	31.6	3.7±0.36	35.4	23.5±1.19	11.5	4.8±0.23	17.3
18	51.5±4.87	42.3	74.3±2.56	15.4	36.5±1.61	19.8	6.0±0.31	23.3	24.5±1.06	18.8	2.0±0.00	0,00
19	26.4±2.00	34.1	34.3±0.84	11.1	36.5±0.96	11.8	6.1±0.42	31.1	22.1±0.86	17.6	2.5±0.11	20.4
20	52.8±4.60	39.0	42.5±1.46	15.4	44.1±1.57	15.9	5.4±0.28	22.8	28.7±1.27	19.9	3.2±0.18	25.3
21	41.2±2.33	25.2	37.4±0.67	8.0	41.5±1.16	12.5	5.8±0.14	10.9	29.5±1.01	15.3	2.7±0.12	21.4
22	33.0±1.88	25.2	38.6±0.85	9.8	37.2±0.77	9.2	5.0±0.13	12.0	24.3±0.89	16.5	2.4±0.13	25.0
Average, V, %		31.2±2.36		12.8±1.24		14.6±1.41		21.0±1.27		18.1±1.13		18.9± 1.26

Cluster analysis (k-means method) demonstrated that the interclusterian variance was much higher than the intraclusterian one for such characters as the fruit mass, fruit height and diameter and mesocarp thickness (Table 2). Thus, studied genotypes showed pronounced differences in this case. In contrary, the interclusterian variance was lower than the intraclusterian one for the pericarp thickness and the number of locules. However, the differences between genotypes based on these characters were insignificant.

Table 2. Analysis of the inter- and intraclusterian variance of some fruit characteristics

Character	Interclusterian variance	df	Intraclusterian variance	df	F	p
Fruit mass	12728.55	3	1524.626	18	50.09182	0.000000
Fruit length	1652.21	3	706.271	18	14.03611	0.000058
Fruit diameter	1290.79	3	365.569	18	21.18544	0.000004
Pericarp thickness	2.82	3	23.602	18	0.71613	0.555132
Mesocarp thickness	1465.69	3	542.697	18	16.20453	0.000024
Number of locules	17.26	3	24.160	18	4.28608	0.018961

Cluster analysis by the k-means method revealed that the groups of genotypes, separated into 4 clusters, differed according to the level and variability of the studied characters (Figure 3, Table 3).

By classifying the genotypes based on the 6 characters, it was found that cluster 1 included 9: Rufina, Charovnita, Viking, Golden Jubilee, MilOrang, Chihlimbar, Hurma, Dolgonosic, L 10B. Cluster 2 included the Timisorean landrace with the highest values of characters. Cluster 3 included Rosinca, Gold Nugget, Flacara, Moldavian landrace, Vrojainii, De-barao yellow. Cluster 4 included Luci, Alex, Breeding Line, Mia, Buyan yellow, Oranjevie sosulki. Pericarp thickness and locules number were factors with lower discriminant capacity in classifying cluster genotypes.

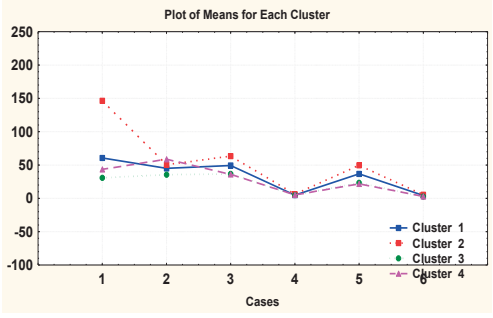


Figure 3. Ability to differentiate clusters (k-means method) using the characteristics of tomato fruit. Horizontal: 1. Fruit mass; 2. Fruit length; 3. Fruit diameter; 4. Pericarp thickness; 5. Mesocarp thickness. 6. Number of locules. Vertical: 1, 2, 3, 4 - clusters of tomato genotypes

Table 3. Descriptive analysis of clusters

Cluster	Character	x	Genotype
1	Fruit mass	60.61	1 – Rufina, 2 – Charovnita, 4 – Viking, 6 – Golden Jubilee, 8 – MilOrang, 12 – Chihlimbar, 13 – Hurma, 16 – Dolgonosic, 20 – L 10B.
	Fruit length	45.06	
	Fruit diameter	49.26	
	Pericarp thickness	4.87	
	Mesocarp thickness	36.79	
	Number of locules	4.33	
2	Fruit mass	146.50	7 – Timisorean landrace
	Fruit length	50.30	
	Fruit diameter	63.50	
	Pericarp thickness	6.40	
	Mesocarp thickness	49.50	
	Number of locules	5.50	
3	Fruit mass	30.78	3 – Rosinca, 5 - Gold Nugget, 11 – Flacara, 19 – Moldavian landrace, 21 – Vrojainîi, 22 – De-barao yellow
	Fruit length	35.65	
	Fruit diameter	36.73	
	Pericarp thickness	4.78	
	Mesocarp thickness	23.45	
	Number of locules	2.60	
Cluster	Character	x	Genotype
4	Fruit mass	43.52	9 – Luci, 10 – Alex, 14 – Breeding Line, 15 – Mia, 17 – Buyan yellow, 18 – Oranjevie sosulki
	Fruit length	58.83	
	Fruit diameter	36.13	
	Pericarp thickness	5.27	
	Mesocarp thickness	21.67	
	Number of locules	2.90	

The number of fruits per plant and the productivity per plant were evaluated in 15 varieties. The results are shown in Fig.4. The number of fruits per plant in the studied group varied within 8-28. More than 20 fruits were

registered in the varieties Rufina, Dolgonisic, Local form (Moldova), Flacara (Figure 4A). The productivity per plant was: 0.186 kg to 0.91 kg. In Figure 4 it can be observed that the productivity of the studied varietie was rather low, because soil-climatic conditions of the year were quite harsh for the cultivation of tomatoes without irrigation.

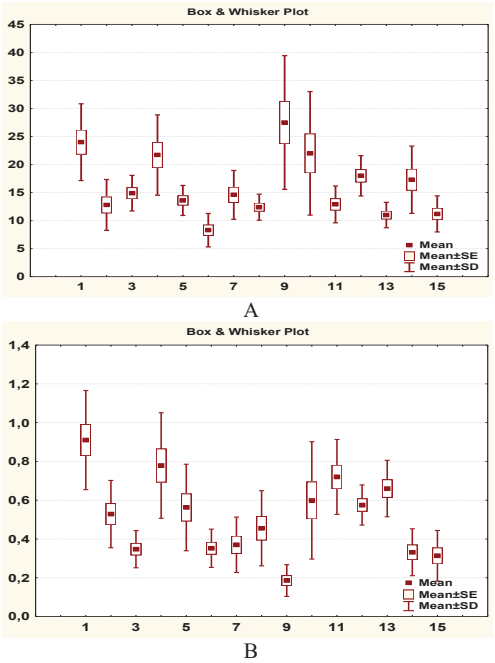


Figure 4. Variability of productivity traits (A - number of fruits per plant, B - productivity per plant, kg.) in tomatoes:
1 – Rufina, 2 – Charovnita, 3 – Dolgonisic, 4 – Rosinca, 5 – Viking, 6 – MilOrang, 7 – Luci, 8 – Alex, 9 – Moldavian landrace, 10 – Flacara, 11 – L 10B (Buzau), 12 – Vrojainii, 13 – Chihlimbar, 14 – De-barao yellow, 15 – Mia

The varieties manifesting a complex of economically valuable characters were tested at 4 temperatures: 25°C - optimal; 38^{0}C, 40^{0}C, and 42^{0}C - stressful. It was found that the root length varied within 26.2-44.8 mm under optimal conditions, while at 38^{0}C - 24.8-51.3 mm (Fig. 5A). The degree of growth inhibition in the varieties Rosinca, Viking, Amber was -0.7; -2.6; -11.4, respectively (compared to optimal conditions). Stimulation was registered in Rufina (11.7%), Luci (11.5%), and Flacara (7.3%). In the case of the temperature of 40^{0}C, the degree of inhibition of the embryonic root growth varied in the ranges of -10.8...-36.8%. There was no stimulation in any of the varieties. A relatively low inhibition was found in the varieties Rufina, Luci and Amber. This indicates the pronounced genetic determination of the root respons to stressful temperatures. The significant inhibition of the root growth was observed in all studied varieties under the temperature of 42^{0}C, with the exception of the variety Luci. In Luci this parameter decreased by 6.5%. The degree of growth inhibition compared to the control was: 59.0% (Rufina); 58.7% (Viking); 57.5% (Flame); 52.1% (Chihlimbar); 42.3% (Rosinca).

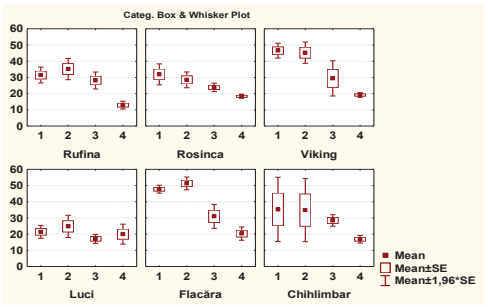
The length of the stem in the control variant varied in the ranges of 16.6-25.9 mm (Figure 5B). Under stressful temperatures, the genotypes showed a differentiated response and high variability of this trait, within 10.5-16.5 mm. The temperature of 40^{0}C significantly inhibited the growth of the stem in 5 genotypes compared to the optimal temperature. It is especially visible in the varieties Viking, Rufina and Flacara, in which the decrease of this parameter was -51.4%, -39.3%, and -39.0%, respectively. The stressful temperature of 42^{0}C inhibited the growth of stem in all studied varieties. Inhibition ranged from -12.3% to -58.6% compared to control. The greatest influence of temperature on the length of the stem was observed in the Viking (58.6%) and Rufina (-57.1%) varieties.

It was found that the temperature of 38^{0}C had a stimulating effect on seedling growth in the varieties Luci (+ 12.1%), Rufina (+ 7.6%) and Flacara (+ 3.8%) (Fig. 4C). In the other three varieties, inhibition of the character was insignificant: -2.7%, -5.4%, and -5.5%. The temperature of 40^{0}C determined a significant decrease in seedling length only in the Viking (-42.1%) and Flacara (-36.6%) varieties. The temperature of 42^{0}C decreased the length in the varieties Viking (-57.4%), Rufina (-56.6%), Flacara (-50.2%) and Rosinca (-39.3%). Therefore, maintaining germinated tomato seeds for 6 hours at 42^{0}C is the most effective treatment for differentiating genotypes by the level of resistance to thermal stress.

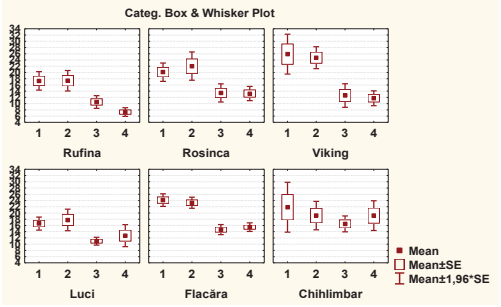
Based on the assessment of tomato genotypes by three test parameters, it can be concluded that the varieties Luci and Chihlimbar have shown complex resistance to thermal stress.

The statistical processing of the experimental data by the bifactorial analysis of the variance allowed the appreciation of the variability and the degree of influence of the temperature, genotype and their interaction on the variability of the evaluated characters (Table 4).

It was found that the contribution of genotype, temperature and genotype × temperature interaction to the growth of the embryonic root of tomato was 23.3%; 70.5%; 4.0%, to the stem growth 18.1%; 76.4%; 3.4%, and to the seedling growth 20.2%; 74,7%; 3.2%, respectively (Table 4). So, the most contributed factor in the growth of the root, stem and seedling is the temperature (70.5%, 76.4% and 74.7%).



A



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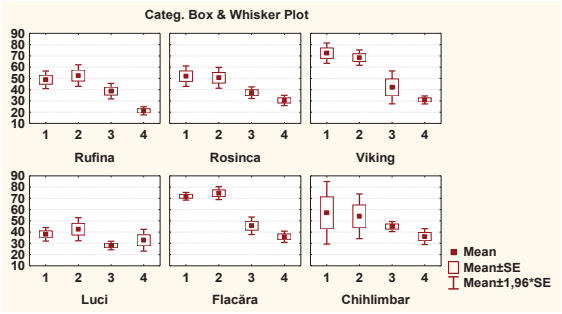

C

Figure 5. Influence of temperature on the length of the root (A), stem (B) and seedling (C) of tomatoes.
Horizontal: 1 = control (25°C); 2 = 38^0C; 3 = 40^0C; 4 = 42^0C

Table 4. Bifactorial analysis of tomato genotype x temperature relationships

Source of variation	Degree of freedom	Root length		Stem length		Seedling length	
		Mean sum of squares	Contribution in the source of variation, %	Mean sum of squares	Contribution in the source of variation, %	Mean sum of squares	Contribution in the source of variation, %
Tomato genotype	5	463.08*	23.3	83.20*	18.1	811.3*	20.2
Temperature	3	1399.40*	70.5	351.90*	76.4	3007.5*	74.7
Tomato genotype x temperature	15	79.26	4.0	15.90	3.4	127.6	3.2
Random effects	48	43.99	2.2	9.68	2.1	78.2	1.9

*- $p<0.05$.

CONCLUSIONS

The tomato varieties carrying the *β* (carotene) and *r* (yellow flesh) genes differ on the base of morpho-biological characters, precocity, fruit characteristics, productivity.

It was found using cluster analysis (Ward method) that studied varieties differ in

similarity of assessed characters such as fruit mass, fruit length, fruit height, pericarp thickness, mesocarp thickness, locule number. The highest similarity was recorded for the varieties Rosinca, Vrojainii, Flacara, De-barao yellow, Moldavian landrace

Cluster analysis (k-means method) of the varieties showed that the interclusterian variance was much higher than the intraclusterian one for the fruit mass, fruit height and diameter, and mesocarp thickness. It means that studied genotypes have clearly pronounced differences. The interclusterian variance was lower than the intraclusterian one for the characteristics of the pericarp thickness and the number of locules. However, in this case the difference between genotypes was insignificant.

It was found that the response of tomato plants (root, stem, and seedling growth) to stressful temperatures was different and depended on the growing organ, genotype and the temperature.

As a result of the bifactorial analysis, it was found that the contribution of the temperature in the variability of tomato growing organs is much higher than the contribution of the genotype.

The varieties Luci and Chihlimbar have manifested a low sensitivity to high temperatures and are therefore of interest in breeding as possible genetic sources of heat resistance.

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