INFLUENCE OF THE TEMPERATURE STRESS ON GROWTH AND DEVELOPMENT CHARACTERS OF THE TOMATO GENOTYPES

MIHNEA Nadejda, LUPAŞCU Galina, RUSU Vadim

Abstract. The paper presents the results of screening of some varieties and 2 reciprocal F_2 tomato hybrid combinations: *Desteptarea x Flacara/Flacara x Desteptarea and Flacara x Vrojainii/Vrojainii x Flacara*, based on resistance to low positive temperatures (10°, 11°C) in early ontogenesis. Seedlings grown at 25ºC served as a control. The analysis of the variability of the resistance character was performed based on *germination, length of the embryonic radicle, stem* and *intact seedling*. In the most cases, low temperatures produced significant inhibition of the both germination and growth organs. By cluster analysis (*k*-means method) in the 11°C variant, the varieties *Chihlimbar, Desteptarea, Flacara, Mary Gratefully* and the reciprocal combination F_2 *Desteptarea x Flacara/* F_2 *Flacara x Desteptarea* were identified, which formed a separate cluster, with the highest values of the evaluated characters. In the case of temperature 10°C – the genotypes/populations *Flacara, Mary Gratefully,* F_2 *Desteptarea x Flacara,* F_2 *Flacara x Desteptarea,* F_2 *Flacara x Vrojainii* presented the cluster with the highest germination values. Research of the influence of low positive temperatures on the distribution of tomato *Solanum lycopersicum* plants into phenotypic classes based on radicle length, demonstrated that the profile of the F_2 reciprocal populations was influenced by both the temperature level and the parental entity when obtaining F_1 reciprocal hybrids.

Keywords: resistance, tomato *Solanum lycopersicum*, temperature, variability.

Rezumat. Influența stresului de temperatură asupra caracterelor de creștere și dezvoltare a genotipurilor de tomate. În lucrare sunt prezentate rezultatele *screening*-uluii unor soiuri și 2 combinații hibride reciproce F_2: *Deșteptarea x Flacăra/Flacăra x Deșteptarea și Flacara x Vrojainîi/ Vrojainîi x Flacara* de tomate în baza rezistenței la temperaturi joase positive (11º, 10ºC) în ontongeneză timpurie. În calitate de variantă martor au servit plantulele cultivate la 25ºC. Analiza variabilităţii caracterului de rezistenţă a fost efectuată în baza germinației, lungimii radiculei embrionare, tulpiniței, plantulei integrale. În majoritatea cazurilor, temperaturile joase au produs inhibare semnificativă atât a germinației cât și a organelor de creștere. Prin analiză clusteriană (*k*-medii) în varianta cu 11ºC au fost identificate soiurile *Chihlimbar, Deșteptarea, Flacăra, Mary Gratefully combinația reciprocă* F_2 *Deșteptarea x Flacăra/* F_2 *Flacăra x Deșteptarea* care au format un cluster separat, cu cele mai înalte valori ale caracterelor evaluate. În cazul temperaturii 10^0C – genotipurile/populațiile *Flacăra, Mary Gratefully,* F_2 *Deșteptarea x Flacăra,* F_2 *Flacăra x Deșteptarea,* F_2 *Flacăra x Urojainîi* au fprezentat clusterul cu cele mai înalte valori ale germinației. Cercetarea influenței temperaturilor joase pozitive asupra repartiției plantelor de tomate *Solanum lycopersicum* în clase fenotipice pe baza lungimii radiculei, a demonstrat că profilul populațiilor reciproce F_2, a fost influențat atât de nivelul de temperatură cât și de factorul parental.

Cuvinte-cheie: rezistență, tomate *Solanum lycopersicum*, temperatură, variabilitate.

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are one of the most important vegetable crops worldwide, due to the high value of the fruits and the various forms of consumption in fresh and industrially processed form (BARONE et al., 2008; GIOVANNONI, 2004).

Temperature is one of the main ecological factors, difficult to predict and regulate, with a significant influence on all vital processes and plant productivity (HOUGHTON & YIHUI, 2001; SNIDER et al., 2012). Recent studies have shown that the intensity, frequency and duration of extreme weather events have changed in recent years, especially extreme temperatures (UMMENHOFER & MEEHL, 2017). As a result, climate change poses major challenges for agriculture worldwide, especially high and low temperatures (MEENA et al., 2018). According to the Intergovernmental Panel on Climate Change (COLLINS et al., 2013), there will be more records of high temperatures than cold temperatures in a warmer climate. Although the phenomenon of global warming is increasingly felt, low temperature stress remains one of the major abiotic factors, which greatly affects cell survival and division (SANGHERA et al, 2011; JENA et al. 2012), germination capacity, seed viability (GIOVANNUCCI et al., 1995), vegetative growth (CHEN et al., 2015; KANAVI et al., 2020), flowering duration, number of flowers and fruits per plant (MEENA, 2018; SHERZOD et al., 2019), photosynthesis intensity [JENA et al. 2012; SHERZOD, 2019). Some studies have shown that heat stress also affects fruit quality, decreasing vitamin C content (XIAOA et al., 2018) and lycopene content, the percentage of lycopene reduction being highly dependent on the variety (VIJAYAKUMAR et al., 2021).

The stress caused by low positive temperatures considerably reduces the resistance of plants to various fungi, especially the causative agents of root rot [LUPASHKU & ROTARU, 2010; LUPASHKU et al., 2020]. Sensitivity to reduced temperatures restricts the geographical spread of the developed varieties. Moreover, in spring the soil, which often has temperatures lower than 15ºC, directly restricts the sowing of tomatoes. The creation of cold-tolerant (CT) varieties offers significant advantages for the production of tomatoes under suboptimal conditions in the field. For example, CT plants grow faster at early stages, becoming well developed much earlier than susceptible ones. This can improve earliness, adaptability, water saving capacity, obtaining high quality fruits under suboptimal growing conditions. Moreover, CT tomato varieties can be planted earlier, thus obtaining earlier harvests when the price of fruits is higher, which brings a real economic profit. The level of production can also be improved because early planting avoids high summer temperatures, which considerably reduce the fruit-setting capacity. Finally, TF contributes to reducing the amount of water used in dry regions because plants that grow and develop early use spring-early summer rains more efficiently and rationally (FOOLAD & LIN, 2001; HU et al., 2006).

The purpose of our research was to evaluate the influence of low temperatures on plant growth and development traits in some varieties, reciprocal combinations of tomatoes, and identify those with potential for use in breeding programs.

MATERIAL AND METHODS

The experiments used: i) 9 tomato *Solanum lycopersicum* varieties, of which 5 (Exclusiv, Prestij, Deşteptarea, Flacăra, Mary Gratefully) – from the collection of the Applied Genetics laboratory of the Institute of Genetics, Physiology and Plant Protection, MSU; ii) 4 – of foreign origin (Pontina, Florina, Chihlimbar, Vrojainîi); iii) 2 reciprocal F_2 hybrid combinations: Desteptarea x Flacara/Flacara x Desteptarea and Flacara x Vrojainii/Vrojainii x Flacara.

In the research, 3 temperature levels were applied: optimal – 25°C and stress: 10°C and 11°C. The assessment of the resistance of tomato samples to low temperatures (10°C and 11°C) was carried out based on germination, growth capacity of the embryonic radicle, stem and intact seedlings after maintaining them for 21 days at stress temperatures (MIHNEA, 2016). Seedlings maintained at a constant temperature of 25°C for 7 days served as controls. Plant resistance (%) was calculated according to the ratio of the values of the investigated traits from the stress variant to the control variant, and tomato samples were differentiated into resistance groups: 81-100% – highly resistant, 61-80% – resistant, 41-60 medium resistant, 21-40% – weak resistance, <20% – non-resistant.

The cluster analysis of the degree of similarity/difference of tomato genotypes based on growth and development characters at different temperatures was performed based on the iterative algorithm for building dendrograms and the *k*-means centroid method - methods successfully used in genetics and breeding research (KANAVI et al., 2020; LUPAŞCU et al., 2019). The obtained data were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSION

Analysis of the reaction of tomato plants to low positive temperatures demonstrated that under the action of temperature stress, in most of the genotypes studied, there was a strong inhibition of seed germination, embryonic radicle length, stem and intact seedlings.

Seed germination analysis showed that tomato genotypes demonstrated a differentiated reaction both under optimal and stress conditions. It was found that in the control variant germination varied within the limits of 75.6...96.7%; at a temperature of 11°C – 38.9...98.8% and at a temperature of 10°C – 0.0...84.4%. It is worth noting that no germinated seeds were recorded in the Pontina variety. The resistance of genotypes to the temperature level of 11°C was within the limits of 51.4...103.9%, and at 10°C – 0...90.5%. Highly resistant to both temperature levels was the Flacara variety, and resistant – Mary Gratefully, F_2 Desteptarea x Flacara, F_2 Flacara x Desteptarea, F_2 Flacara x Vrojainii. At the temperature of 11°C no non-resistant genotypes were recorded, but they were recorded at a temperature of 10°C – Pontina, Florina, Exclusiv, Vrojainîi (0 ... 36.3%). The radicle, stem and seedling of tomatoes also showed a high sensitivity, but at the same time specific to thermal stress. It was found that at a temperature of 11°C the degree of inhibition of embryonic radicle growth varied in the range of 67.8-88.1%; stem – 62.8…88.7 and seedling 61.1…85.5%, and at a temperature of 10°C – 87.6…95.6; 86.4…96.9; 89.4…94.8%, respectively, of the examined characters (Fig. 1).

The classification of evaluated genotypes in clusters according to the values of growth and development characters – small, medium, large, demonstrated that the thermal factor had an important role in differentiating genotypes. Cluster analysis (*k*-means method) demonstrated that for the length of the radicle, stem, seedling, in the control variant the intercluster variance was much higher than the intracluster, while for germination the intercluster variance was much lower than the intracluster. This denotes that the studied 10 genotypes showed pronounced differences based on the length of the radicle, stem, seedling and less in terms of seed germination (Table 1).

By classifying the genotypes based on the evaluated characters, it was found that in the variant with the temperature regime of 11°C the varieties Cihlimbar, Deșteptarea, Flacăra, Mary Gratefully, F_2 Deșteptarea x Flacara, F_2 Flacara x Desteptarea formed a cluster with the highest values of seed germination and growth characters, and at the temperature of 10°C no stable groups of genotypes were found for all characters, which denotes the manifestation of a pronounced specific plasticity of germination and growth organs in response to temperature (Table 2). The highest germination values were recorded by Flacara, Mary Gratefully, F_2 Desteptarea x Flacara, F_2 Flacara x Desteptarea, F_2 Flacara x Vrojainii, the average being 70.2%. In terms of radicle length, the varieties Chihlimbar, Prestij, Desteptarea and the hybrid combination F_2 Vrojainii x Flacara had an advantage of 34.2%, with an average germination of 50.8%. The use of these genotypes by increasing the seeding rate could contribute to a more uniform emergence of seedlings. It was found that under thermal stress, the intercluster variance was much higher than the intracluster variance, especially for seed germination. In the case of radicle, stem and seedling length, the intercluster variance was higher than the intracluster variance in the 11°C temperature variant, while at 10°C the difference between these parameters had no statistical support, which denotes the weak differentiation of genotypes in clusters. It is worth noting that the germination faculty presented a relevant criterion for differentiating the tested forms for both stress temperatures (Table 2).

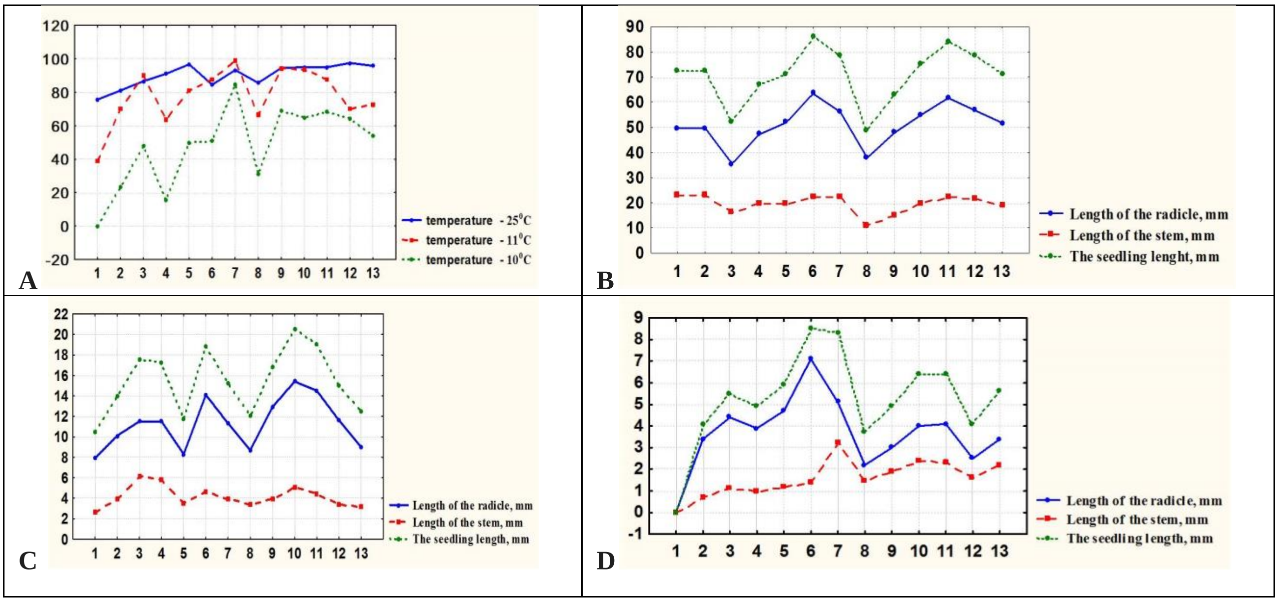


Figure 1. Germination capacity (%) – A and growth of tomato seedlings at different temperatures – B (25°C), C (11°C), D (10°C)
1 – Pontina, 2 – Florina, 3 – Chihlimbar, 4 – Exclusiv, 5 – Prestij, 6 – Desteptarea, 7 – Flacara, 8 – Vrojainii, 9 – Mary Gratefully, 10 – F_2 Desteptarea x Flacara, 11 – F_2 Flacara x Desteptarea, 12 – F_2 Flacara x Vrojainii, 13 – F_2 Vrojainii, x Flacara.

Table 1. Analysis of inter- and intracluster variance in the interaction of tomato genotypes with temperature.

Variant	Intercluster variance	df	Intracluster variance	df	F	p
25°C						
Length of the radicle	653,621	2	127,9668	10	25,53869	0,000118
Length of the stem	85,471	2	72,1572	10	5,92253	0,020102
The seedling length	1183,355	2	281,2760	10	21,03547	0,000261
Germination	219,663	2	346,6243	10	3,16861	0,085923
11°C						
Length of the radicle	47,488	2	24,1817	10	9,81892	0,004373
Length of the stem	4,699	2	8,1933	10	2,86756	0,103669
The seedling length	79,671	2	39,6367	10	10,05017	0,004047
Germination	3036,144	2	280,7883	10	54,06465	0,000004
10°C						
Length of the radicle	12,784	2	20,5595	10	3,10892	0,089132
Length of the stem	4,928	2	3,3955	10	7,25604	0,011301
The seedling length	25,249	2	30,7230	10	4,10919	0,049826
Germination	6207,129	2	819,7198	10	37,86129	0,000022

Table 2. Descriptive cluster analysis.

Clus-ter	Caracter	Control (25°C)		Temperature 11°C		Temperature 10°C	
		Mean	Genotype/ combination	Mean	Genotype/ combination	Mean	Genotype/ combination
1	Length of the radicle, mm	36.75	3, 8	9.87	2, 4, 5, 8, 12, 13	2.37	1, 2, 4, 8
	Length of the stem, mm	13.70		3.87		0.80	
	The seedling length, mm	50.40		13.78		3.17	
	Germination, %	86.10		70.60		17.50	
2	Length of the radicle, mm	56.67	5, 6, 7, 10, 11, 12, 13	13.28	3, 6, 7, 9, 10, 11	4.90	3, 5, 6, 13
	Lnegth of the stem, mm	21.06		4.70		1.47	
	The seedling length, mm	77.71		17.98		6.37	
	Germination, %	93.97		92.02		50.77	
3	Length of the radicle, mm	48.62	1, 2, 4, 9	7.90	1	3.74	7, 9, 10 , 11, 12
	Length of the stem, mm	20.10		2.60		2.28	
	The seedling length, mm	68.77		10.50		6.02	
	Germination, %	85.55		38.90		70.16	

Note: genotype/combination number – according to Fig. 1.

By calculating the percentage weight in the source of variation (WSV) of characters, it was found that the contribution of genotype, temperature and *genotype x temperature* interactions in radicle length was 0.42; 99.30; 0.22%; stem – 3.52; 96.03; 0.37%, seedling – 0.37; 99.34; 0.23% and germination – 9.14; 88.44; 1.76%.

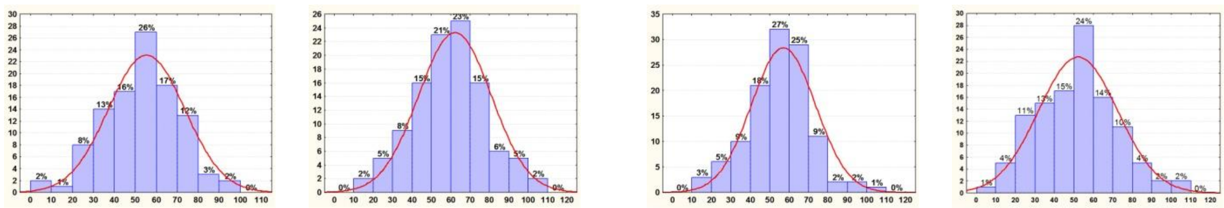
So, through factorial analysis it was found that the weight of temperature in the source of variation of germination, radicle, stem and seedling length was the highest, which denotes that the temperature factor is the most important discriminatory factor for the evaluated characters. From the presented data it is observed that in general, the role of genotype (0.7-9.14%) and *genotype x temperature* interactions (0.22-1.76%) were less important for the growth of tomato seedlings (Table 3).

Table 3. The contribution of genotype and temperature to the growth capacity of tomatoes.

Source of variation	**Freedom degree**	**The length of the radicle**		**The length of the stem**		**Length of the seedling**		**Germination**	
		Mean sum of squares	**WSV, %**	**Mean sum of squares**	**WSV, %**	**Mean sum of squares**	**WSV, %**	**Mean sum of squares**	**WSV, %**
Genotype	12	115,6*	0,42	147,7*	3,52	194,4*	0,37	2058,7*	9,14
Temperature	2	27440,2*	99,30	4029,5*	96,03	52583,7*	99,34	19913,1*	88,44
Genotype x temperature	24	60,8*	0,22	15,5*	0,37	119,5*	0,23	395,2*	1,76
Random effects	90	17,9	0,06	3,5	0,08	32,9	0,06	148,3	0,66

*- $p<0,05$.

Analysis of the distribution histograms of tomato plants under ***optimal temperature*** conditions according to radicle length for populations obtained based on the reciprocal hybrids F_1: F_2 Desteptarea x Flacara / F_2 Flacara x Desteptarea demonstrated essential differences. If in the combination Desteptarea x Flacara the maximum of the distribution frequencies was located in the phenotypic class 50-60 mm (28%), then in F_2 Flacara x Desteptarea – in the class 60-70 mm (23%). The class with maximum values – 90-110 mm constituted 2% for F_2 Desteptarea x Flacara, and for F_2 Flacara x Desteptarea the class with maximum values was 100-110 mm and constituted 5%. Regarding the reciprocal combination F_2 Flacara x Vrojainii / F_2 Vrojainii x Flacara, the maximum frequency distribution in both combinations was represented by the phenotypic class 50-60 mm, being 27 and 24% respectively, while in the phenotypic class with higher values – 80-110 mm in the combination F_2 Vrojainii x Flacara the number of genotypes was twice as high compared to F_2 Flacara x Vrojainii, being 8 and 4% respectively (Fig. 2).



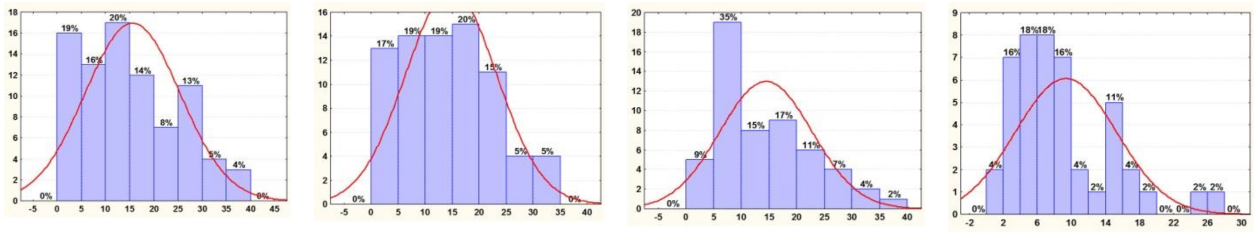
F_2 Desteptarea x Flacara F_2 Flacara x Desteptarea F_2 Flacara x Vrojainii F_2 Vrojainii x Flacara

Figure 2. Histograms of tomato plant distribution based on radicle length in reciprocally segregating populations under optimal conditions (25°C).

Note: *Vertically* – The number of plants. *Horizontally* – Phenotypic classes (mm).

By investigating the influence of the ***temperature of 11°C*** on the distribution of plants into phenotypic classes, it was found that in the F_2 Deşteptarea x Flacăra population there were 3 maxima of the distribution of values: 0-5 mm (19%), 10-15 (20%), 25-30 (15%), which denotes the decomposition of the population into 3 subpopulations with their own characteristics. In the F_2 Flacăra x Deşteptarea combination such a phenomenon was not recorded. It is worth noting that the number of genotypes with maximum values in the first combination was 35-40 mm (4%), and in the 2nd – 30-35 mm (5%). Regarding the F_2 Flacara x Vrojainîi, F_2 Vrojainîi x Flacara combinations, it was found that at a temperature of 11°C, in both combinations the maximum of the frequency distribution was shifted to the left, in the direction of low values. At the same time, the frequency of genotypes with higher indices (from the theoretical center of the distribution of values to the right) constituted 41% for the first combination and 27% – for the second. As in the case of the combination F_2 Desteptarea x Flacara, in F_2 Vrojainii x Flacara the decomposition of the initial population into subpopulations occurred, which presents a phenotypic marker of sensitivity to temperature stress at the population level (Fig. 3).

A similar phenomenon of population decomposition was observed in common wheat genotypes, sensitive to the action of the biotic factor – the causal agents of root rot (LUPAȘCU et al., 2015). However, the probability of obtaining a greater number of segregants with reduced sensitivity to the temperature of 11°C belongs to the combinations F_2 Desteptarea x Flacara and F_2 Flacara x Vrojainii.

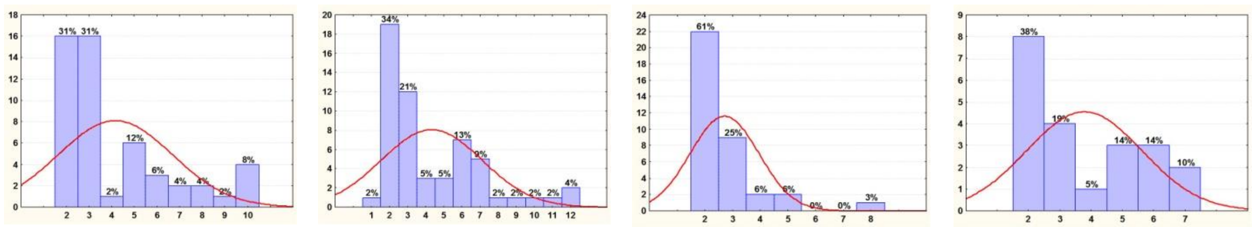


F_2 Desteptarea x Flacara F_2 Flacara x Desteptarea F_2 Flacara x Vrojainii F_2 Vrojainii x Flacara

Figure 3. Histograms of tomato plant distribution based on radicle length in reciprocally segregating populations under stress conditions (11ºC).

Note: *Vertically* – The number of plants. *Horizontally* – Phenotypic classes (mm).

Regarding the influence of the ***10ºC temperature***, it can be observed that in both reciprocal combinations the maximum of the frequency distribution compared to the optimal conditions was shifted to the left. In the F_2 Flacara x Desteptarea combination the rate of plants that developed embryonic radicles with a length of 6-12 mm was much higher than in its counterpart, and in the reciprocal F_2 Flacara x Vrojainii / F_2 Vrojainii x Flacara combination a greater number of segregants with reduced sensitivity to the temperature of 10ºC belongs to the combination in which the paternal form was the Flacara variety (Fig. 4).



F_2 Desteptarea x Flacara F_2 Flacara x Desteptarea F_2 Flacara x Vrojainii F_2 Vrojainii x Flacara

Figure 4. Histograms of tomato plant distribution based on radicle length in reciprocally segregating populations under stress conditions (10ºC)

Note: *Vertically* – The number of plants. *Horizontally* – Phenotypic classes (mm).

CONCLUSIONS

It was found that low positive temperatures significantly influence the early ontogeny of tomato varieties and reciprocal hybrids by decreasing germination and strongly repressing radicle, stem and seedling growth.

Cluster analysis using the *k*-means method showed that the optimal temperature exhibited a high discriminative capacity of tomato clusters for seedling and radicle length, and at stress temperatures (10, 11°C) – for seed germination, which reveals a more pronounced interaction specificity with these temperatures.

Through factor analysis, it was found that the greatest influence in the source of variation in the length of the radicle, stem, seedling and seed germination was temperature, its contribution constituting 99.3%, 96.03%, 99.34%, 88.44, respectively.

Research into the influence of low positive temperatures on the distribution of plants into phenotypic classes through distribution histograms demonstrated that the spectrum of phenotypic classes for the radicle length trait in early ontogenesis in F_2 populations was influenced by both the temperature level and the parental entity (maternal/paternal) when creating F_1 reciprocal hybrids.

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