IDENTIFICATION OF THE TOMATO GENOTYPES RESISTANT TO THERMAL STRESS USING FACTORIAL AND CLUSTER ANALYSIS

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Abstract. The paper presents the results of assessing the resistance of some genotypes selected from the mutual combination F_2 Prestij x Pontina/ F_2 Pontina x Prestij of tomato to stressful temperatures (42°, 10°C). Seedlings grown at 25ºC served as a control variant. The analysis of the variability of the resistance character was carried out based on the length of the embryonic radicle, stem, whole seedling and germination. In most cases, stressful temperatures caused significant inhibition of growth organs. Through cluster analysis (*k*-means method), the parents – 204/I/2 pl. F_3 Prestij x Pontina and Prestij were identified, with the highest values of the radicle, stem and seedling length characters at temperature 42°C and 205/II/3 pl. F_3 Pontina x Prestij, 205/II/5 pl. F_3Pontina x Prestij, Pontina that showed increased resistance based on seed germination at 10°C, which offers opportunities to use them in breeding programs as reliable sources of resistance.

Keywords: tomato, genotypes, temperature, factorial analysis, cluster analysis.

Rezumat. Identificarea genotipurilor de tomate rezistente la stresul termic cu ajutorul analizelor factorială și clusteriană. În lucrare sunt prezentate rezultatele aprecierii rezistenței unor genotipuri selectate din combinația reciprocă F_2 Prestij x Pontina / F_2 Pontina x Prestij de tomate la temperaturi stresante (42°, 10°C). În calitate de variantă martor au servit plantulele cultivate la 25ºC. Analiza variabilităţii caracterului de rezistenţă a fost efectuată în baza lungimii radiculei embrionare, tulpiniței, plantulei integrale și germinației. În majoritatea cazurilor, temperaturile de stres au produs inhibarea semnificativă a organelor de creștere. Prin analiză clusteriană (metodă *k*-medii) au fost identificați genitori – 204/I/2 pl. F_3 Prestij x Pontina și Prestij cu cele mai înalte valori ale caracterelor lungimea radiculei, tulpiniței și plantulei la temperatură 42°C și 205/II/3 pl. F_3 Pontina x Prestij, 205/II/5 pl. F_3 Pontina x Prestij, Pontina care au manifestat o rezistență sporită conform germinației semințelor la temperatură 10°C, ceea ce oferă oportunități de utilizare a acestora în programele de ameliorare în calitate de surse sigure de rezistență.

Cuvinte cheie: tomate, genotipuri, temperatură, analiză factorială, analiză clusteriană.

INTRODUCTION

Abiotic stress significantly affects the vegetative and reproductive growth of plants (BOOTE et al., 2012; IBUKUN & KELLY, 2020; FAHRIZAL et al., 2022). Although tomatoes are grown in different ecological-geographic areas, which would demonstrate their high adaptability to diverse climatic conditions, they are still extremely sensitive to high (ALAM et al., 2010) and low temperatures (BARRERO-GIL et al., 2016; YANG et al., 2021).

According to the authors Singh, Prasad, Reddy (SINGH et al., 2013), high temperature is considered one of the factors with the most serious repercussions on agriculture. High temperatures affect plant growth and reproductive organs, leading to significant productivity losses. It should be noted that the reproductive period is more sensitive to thermal stress than the vegetative period (MITTLER & BLUMWALD, 2010). Increasing the temperature by a few degrees compared to the optimal level can greatly affect the reproductive organs, which determines pollen sterility/viability, gamete development and pollination capacity, flower drop and reduced fruit firmness (SATO, 2000; OZORES-HAMPTON et al., 2012). High temperatures can also cause significant productivity losses and damage to fruit quality (NAHAR & ULLAH, 2011; OZORES-HAMPTON et al., 2012; IBUKUN & KELLY, 2020).

Low temperature affects crop productivity by influencing different stages of growth and development in tomatoes. Tomato plants are strongly affected by temperatures between 0 and 15 degrees at all stages of growth. The severity of the effects of cold stress is seen in the decrease of the growth rate (LIU et al., 2012; CHEN et al., 2015), the decrease of the germination capacity (GIOVANNUCCI, 1999), the cellular turgorescence (BLOOM et al., 2004), the intensity of photosynthesis (ZHOU et al., 2012), as well as the significant reduction in plant growth rate (LIU et al., 2012; CHEN et al, 2015) flowering duration (ADAMS et al., 2001; SHERZOD et al, 2019), the number of flowers (SHERZOD et al., 2019) and the number of fruits per plant (SHERZOD et al., 2019). Low temperature also affects fruit quality by reducing the content of vitamin C and lycopene in fruits (XIAOA et al., 2018).

It should be noted that, due to its quantitative nature, resistance depends on a series of factors, among which we can mention genotypic, environmental factors and *genotype x environment* interactions. Large and stable harvests of agricultural crops can be obtained only on the basis of varieties with high productivity potential and high resistance to the unfavorable factors of the external environment.

The purpose of our research was to evaluate the influence of thermal stress on the growth and development characters of plants selected from F2 tomato hybrid populations and to identify promising ones for exploitation in breeding programs.

MATERIAL AND METHODS

As initial material for the research, two tomato parents were used – Pontina, Prestij and 13 forms selected from the reciprocal F_2 combinations Prestij x Pontina, F_2 Pontina x Prestij that showed a series of economically valuable characters in the 2021 year. Based on experience, the mentioned forms are numbered in order: 1 – 204/I/1 F_3 Prestige x Pontina, 2 – 204/I/2 F_3 Prestige x Pontina, 3 – 204/I/6 F_3 Prestige x Pontina, 4 – 204 /II/1 F_3 Prestige x Pontina, 5 – 204/II/3. F_3 Prestige x Pontina, 6 – 204/II/4 F_3 Prestige x Pontina, 7 – 205/I/1 F_3 Pontina x Prestige, 8 – 205/I/2 F_3 Pontina x Prestige, 9 – 205/II/1 F_3 Pontina x Prestige, 10 – 205/II/2 F_3 Pontina x Prestige, 11 – 205/II/F_3 Pontina x Prestige, 12 – 205/II/4 F_3 Pontina x Prestige, 13 – 205/II/5 F_3 Pontina x Prestige, 14 – Pontina, 15 – Prestige.

The selected parents and forms were tested at 3 temperature levels: optimal – 25°C and stress: 42°C and 10°C. The parental forms Pontina and Prestij served as reference genotypes.

The assessment of the resistance of the tomato samples to high temperatures was carried out according to the growth capacity of the embryonic radicle, the stem and the intact seedling for 7 days after maintaining them on day 4 at a temperature of 42°C within 6 hours, and at a temperature of 10°C respectively, for 21 days (MIHNEA, 2016). Seedlings kept constant at 25°C served as a control.

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 an iterative algorithm for building dendrograms and the *k*-means centroid method – successfully used in genetics and breeding research (LUPAȘCU et al., 2019; KANAVI et al., 2020). The obtained data were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

Testing the reaction of plants to the action of stress temperatures showed that, in most cases, the inhibition of the growth of the embryonic radicle, the stem, occurred, which led to the reduction of the length of the intact seedling (Fig. 1).

It was found that the temperature of 42°C in most cases inhibited the growth of the radicle. Only in 3 cases: 3, 12, 14 were stimulations recorded – by 4.4...40.9% compared to the control. In the other cases, an inhibition of 10.6...35.9% was attested. A strong inhibition was found in genotypes 1 (35.9%), 6 (33.7%), 7 (32.5%) and 10 (33.7%). Samples 4, 8, 9 were the most resistant, the radicle length being decreased by 16.8, 10.6 and 18.7%, respectively, in relation to the optimal temperature (Fig. 1A).

For the stem, growth inhibition was as significant as for the radicle, and in some cases even stronger. Only in genotypes 3 and 11, stimulation was recorded by 51.4 and 23.4% under the influence of temperatures of 42°C. In the other variants, the inhibition varied within the limits of 4.3...61.4%. Genotype 1 was the most sensitive, and more resistant – 4 and 12, whose deviation from the control was -61.4; -4.3 and -8.3% respectively (Fig. 1B).

In the case of seedling length, it was found that the temperature of 42°C produced stimulation in genotypes 3 and 12 – by 43.0; 27.9%, respectively. In the other variants, the seedling length constituted 55.9...93.3% of the variant with optimal temperature (25° C) (Fig. 1C).

At the temperature of 10°C, a strong inhibition of both germination and growth organs under study was observed, for which deviations were recorded from the control variable by -11.4...-80.2% – germination; -83.7...-95.8% – radicle length; -66.7...-94.1 – stem length and -74.6...-93.9% – seedling. Significant germination inhibition was observed in genotypes 5 (61.6%), 12 (76.6%), 8 (80.2%). Prestij (-29.0%), 10 (-27.0%), 13 (-22.9) and Pontina (-11.4%) were less sensitive, the resistance being 71.0; 73.0; 77.1; 88.6% respectively (Fig. 1D).

Cluster analysis by constructing the genotypes distribution dendograms, as classification indices serving radicle length, stem length and seedling length, demonstrated that the degree of similarity of tomato samples at different temperature levels was different, both under optimal and stress conditions (Fig. 2).

The distribution based on Euclidean distances highlighted the formation of distinct clusters within the evaluated set of genotypes, which denotes the manifestation of similarities or differences of the genotypes under study, although this phenomenon is different at different levels of aggregation. For example, in optimal conditions (Fig. 2A), samples 8, 9, 13, recorded the highest similarity, confirmed by the smallest Euclidean distances. In the case of temperatures of 42°C and 10°C, the mentioned samples were distributed in different clusters which denotes their specific reaction to the limiting temperatures.

The cluster analysis (*k*-means method) demonstrated that, for all the studied characters, both in the control variant and at the temperature of 42°C, the intercluster variance was much higher than the intracluster variance, which reveals the strong difference between the clusters based on the tested character lot. Unlike these thermal regimes, at the temperature of 10°C, the intercluster variance was lower than the intracluster one in the case of the first 3 characters, except for the seed germination (Table 1). This is evidence that, at low temperature (10°C), the tested tomato samples differed strongly only on the basis of germination capacity, and seedling growth and development was inhibited to almost the same extent in all samples.

By classifying the genotypes based on the assessed characters, it was found that, in the control variant, cluster 3 was formed by 5 genotypes – 3, 7, 11, 12, 14 which recorded the lowest values of the growth characters, but with a germination of 86 .7%, and cluster 1 – the genotypes with the highest values of growth organs, germination constituting 76.0% (Table 2). In the variant with the 42°C temperature regime, 2 genotypes – 2, 15, and in the case of the 10°C temperature, 10 of the mentioned genotypes – 1, 2, 3, 4, 5, 6, 7, 9, 10, 15 formed cluster 2, with the highest values of growth characters. It should be noted that the genotypes in cluster 3 (11, 13, 14) showed increased resistance based on seed germination at 10°C, the average being 71.7%. Based on the obtained data, we can conclude that genotypes 2 and 15 show complex resistance to the mentioned limiting temperatures and are of interest in their use as sources of resistance to unfavourable temperatures (Table 2).

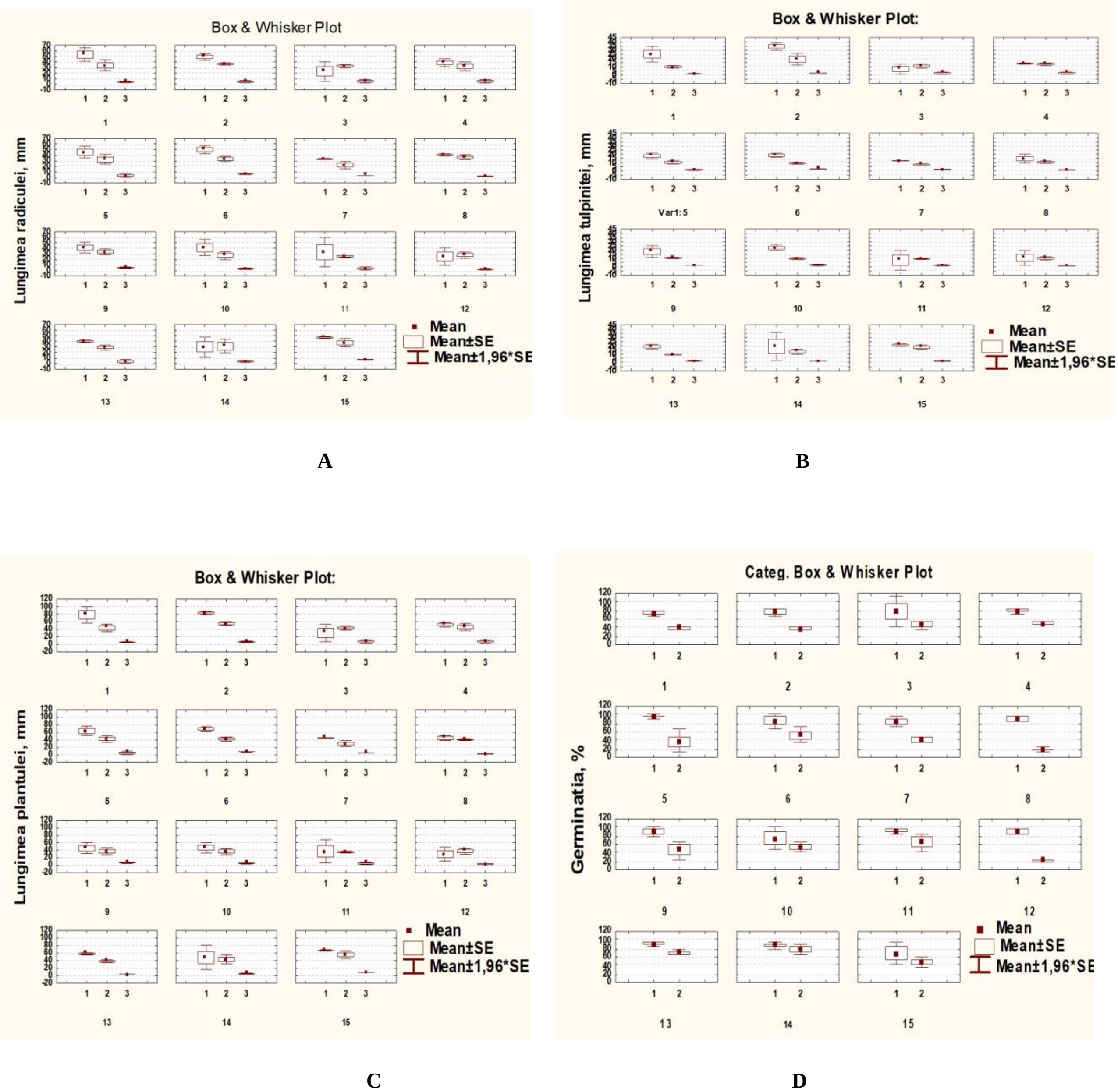
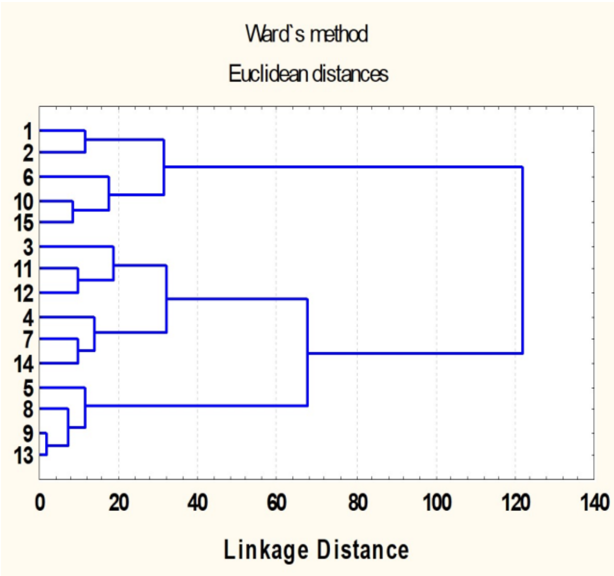
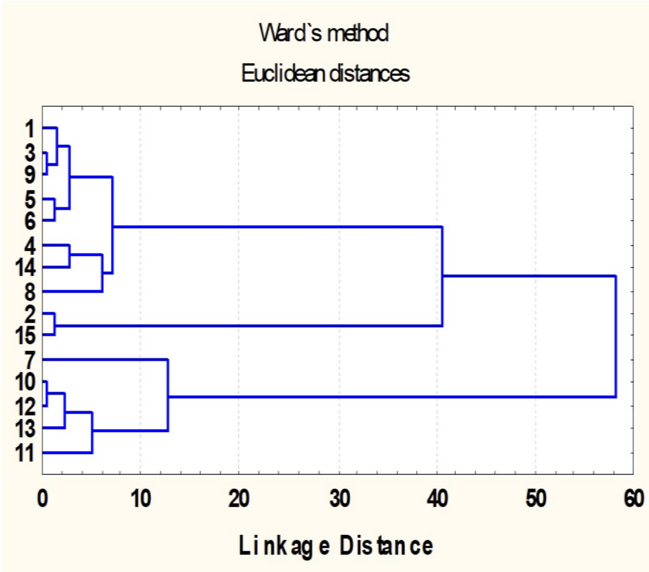


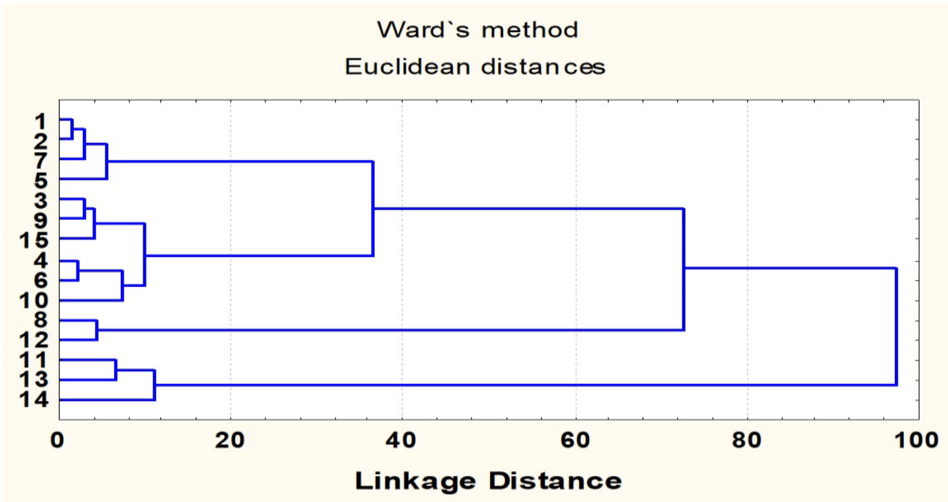
Figure 1. The influence of temperature on some growth and development characters in tomato.
Horizontally – A, B, C: 1 – control (25° C), 2 – 42°C, 3 – 10°C; D: 1 – control (25°C), 2 –10°C.



A



B



C

Figure 2. Dendrogram of distribution of tomato genotypes based on growth characters in optimal conditions A (25°C) and stress B (42°C), C (10°C).

Table 1. Inter- and intracluster variance analysis to the interaction of tomatoes with temperature.

Character	Intercluster variance	df	Intracluster variance	df	F	p
25°C						
The length of the radicle	952,212	2	188,772	12	30,266	0,000
The length of the stem	426,244	2	264,216	12	9,679	0,003
The length of the seedlings	2606,044	2	562,140	12	27,816	0,000
Germination	520,972	2	417,084	12	7,495	0,008
42°C						
The length of the radicle	219,505	2	43,188	12	30,495	0,000
The length of the stem	130,023	2	16,047	12	48,616	0,000
The length of the seedlings	611,546	2	63,064	12	58,184	0,000
10°C						
The length of the radicle	16,072	2	13,765	12	7,006	0,010
The length of the stem	0,727	2	1,111	12	3,926	0,049
The length of the seedlings	18,184	2	28,152	12	3,876	0,050
Germination	3202,764	2	435,500	12	44,125	0,000

Table 2. Descriptive cluster analysis.

Cluster	Caracter	x	Genotype	x	Genotype	x	Genotype
		25°C		42°C		10°C	
1	The length of the radicle, mm	48,20	1, 2, 6, 10, 15	33,33	1, 3, 4, 5, 6, 8, 9, 14	1,95	8, 12
	The length of the stem, mm	24,40		10,94		1,30	
	The length of the seedlings, mm	72,60		44,26		3,25	
	Germination, %	76,00				20,00	
2	The length of the radicle, mm	41,30	4 ,5, 8, 9, 13	37,15	2, 15	4,90	1, 2, 3, 4, 5, 6, 7, 9, 10, 15
	The length of the stem, mm	16,68		18,60		1,96	
	The length of the seedlings, mm	57,98		55,75		6,50	
	Germination, %	41,30				46,10	
3	The length of the radicle, mm	28,94	3, 7, 11, 12, 14	26,42	7, 10, 11, 12, 13	3,60	11, 13, 14
	The length of the stem, mm	11,42		9,18		1,87	
	The length of the seedlings, mm	40,36		35,60		5,47	
	Germination, %	86,66				71,10	

By calculating the percentage weight in the source of character variation, it was found that the contribution of genotype, temperature and *genotype x temperature* interactions in radicle length was 1.0; 97.2; 0.5%; stem – 2.9; 95.0; 1.6%, seedlings – 1.7; 97.2; 0.8% and in germination – 1.6; 96.3; 1.6%.

Table 3. Factorial analysis of *tomato x temperature* relationships.

Source of variation	Freedom degree	Mean sum of squares	Contribution to the source of variation, %
The length of the radicle			
Genotype	14	158,3*	1,0
Temperature	2	15360,6*	97,2
Genotype x temperature	28	74,1	0,5
Random effects	90	53,1	0,3
The length of the stem			
Genotype	14	85,1*	2,9
Temperature	2	2773,3*	95,0
Genotype x temperature	28	47,3*	1,6
Random effects	90	15,2	0,5
The length of the seedlings			
Genotype	14	495,5*	1,7
Temperature	2	27816,6*	97,2
Genotype x temperature	28	230,2*	0,8
Random effects	90	87,4	0,3
Germination			
Genotype	14	491,9*	1,6
Temperature	2	30132,8*	96,3
Genotype x temperature	28	493,3*	1,6
Random effects	90	167,9	0,5

*- p<0,05.

Thus, through factorial analysis, it was found that the weighting of temperature in the source of variation of germination, radicle, stem and seedling length was the highest, which indicates that the temperature factor is decisive for the evaluated characters. From the presented data it can be observed that, in general, the role of genotype (1.0-2.9%) and *genotype x temperature* interactions (0.5-1.6%) was not relevant for the growth of tomato seedlings (Table 3). However, based on one of the integral indices – seedling length, we can observe in the selected forms 204/I/6 F_3 Prestige x Pontina (3), 205/I/1 F_3 Pontina x Prestige (7), 205/II/1 F_3 Pontina x Prestige (9), 205/II/F_3 Pontina x Prestige (11), 205/II/4 F3 Pontina x Prestige (12), relatively small differences between variants 25°C, 42°C, 10°C, which denotes their advanced adaptability to limiting temperatures (Fig. 1).

CONCLUSIONS

The analysis of the growth characteristics – radicle, stem, seedling and germination in the tomato forms selected from the reciprocal F_2 combinations Prestij x Pontina, Pontina Prestij, at different temperature levels, highlighted the differentiated nature of their reaction to limiting temperatures. In most cases, stressful temperatures produced significant inhibition of growth organs in tomato.

Through cluster analysis (*k*-means), 2 samples were identified – 204/I/2 F_3 Prestij x Pontina and Prestij which recorded the highest values of the radicle, stem and seedling length characters at temperature 42°C and 3 samples – 205/II/3 F_3 Pontina x Prestige, 205/II/5 F_3 Pontina x Prestige, Pontina with increased resistance (based on seed germination) at 10°C, which gives opportunities to use them in breeding programs as reliable sources of resistance.

The factorial analysis of the *genotype x temperature* interaction based on testing the reaction of the embryonic radicle, stem, seedling and germination of tomatoes at three temperature levels demonstrated that, in the source of character variation, the main role belongs to the temperature factor (96.3...97, 2%), followed by genotype factors (1.0...2.9%) and *tomato x temperature* interaction (0.3...0.5%). The data show that temperature is the most important factor for the growth and development of tomato plants. However, tomato forms were identified in which there were relatively small differences at the level of the whole plant between the optimal variant (25°C) and the variants with heat stress (10°C, 42°C), which denotes their adaptability to high temperatures.

ACKNOWLEDGEMENTS

The researches were carried out within the project 23.70105.5107.04 "Identification and valorization of valuable parents of agricultural crops in the creation of a native genetic base of socio-economic interest", financed by the National Agency for Research and Development.

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Received: March 06, 2024
Accepted: June 25, 2024