*Original Article*

# Features of the Manifestation of Morphological Traits of Tomato Vegetative and Generative Pollen Cell Nuclei in Lines and  $F_1$  Hybrids Under the Influence of High and Low Temperatures

## Milania MAKOVEI

*Moldova State University, Institute of Genetics, Physiology and Plant Protection, 60, Alexei Mateevici str., MD 2009, Chisinau, Republic of Moldova.*

*Corresponding Author : m\_milania@mail.ru*



*Abstract - The paper presents the results of the studies on the manifestation and variability pattern of morphological traits*  (nucleus diameter, perimeter, area) of vegetative (V) and generative (G) tomato pollen cells in parent lines ( $P_1$  and  $P_2$ ) and their *F*<sub>1</sub> *hybrids under optimal* (25 °C), *high* (45 °C) *and low temperatures* (+7 °C). It has been shown that the nuclei of V and G *pollen cells of different genotypes reacted ambiguously to the action of high and low temperatures. The V pollen cell nuclei were morphologically more homogeneous in the F<sup>1</sup> populations than in their parental forms. On the contrary, the G pollen cell nuclei in the F<sup>1</sup> populations showed high heterogeneity, especially against the background of stresses. The degree of variability of the morphological traits of the nuclei of vegetative (V) and generative (G) pollen cells in F<sup>1</sup> hybrids depends on both the genotype characteristics of their parental forms and the types of stress factors (45 °C and +7 °C). It was found that the indices of the traits of the nuclei of V cells of the pollen of F<sup>1</sup> hybrids in all variants (25 ºС, 45 ºС, +7 ºС) were controlled by the influence of paternal components. While the G pollen cell nucleus traits in F<sup>1</sup> were under the control of paternal forms in optimal conditions (25 ºС), the degree of expression traits nucleus diameter, perimeter and area was under the influence of maternal forms when exposed to high and low temperatures (45 ºС and +7 ºС.)*

*Keywords - F<sup>1</sup> hybrids and their parental lines, Pollen, Tomato, Temperature factors, Vegetative and generative cell nucleus, Variability.*

## **1. Introduction**

The realization of the potential productivity of varieties, lines or hybrids in specific environmental conditions largely depends on their resistance to environmental stress factors (Kang, 2004). The combination of complex genetic systems in one genotype that ensures high productivity and stability is a very difficult genetic breeding task that requires the development of fundamentally new non-traditional methods and approaches, including various methods of pollen breeding. These methods should be aimed at a comprehensive study of environmental sustainability. Identification of variability patterns not only of economically valuable traits under the influence of various stress factors but also the study of morphobiological and cytochemical processes occurring in a plant organism at different stages of ontogenesis and affecting the formation of adaptive reactions and productivity are important in the selection process. One of these stages of plant development includes the stage of the mature male gametophyte, which, according to Pfahler (1982), should be

given primary importance when considering the influence of evolutionary forces acting in populations of higher plants. Male gametes are evolutionarily less protected than female ones, which are closed from direct influences by thick layers of somatic tissues and, therefore, are more sensitive to the action of various stressogens. Adverse environmental conditions affect almost all stages of the formation and development of the male gametophyte (Mesihovic et al., 2016). The results of studies related to the environmental control of pollen formation and development in the early stages show that stress covers many biological processes that lead to disruption of physiological, cytochemical and biochemical mechanisms. As a result, morphological and structural changes occur in the development of pollen grains, a decrease in its fertility and zygotic potential, which are reflected in the level and quality of the resulting crop and seeds (Ettore Pacini et al., 2016). The degree of disturbance of cytological and biochemical processes, which are closely interrelated, depends on both the type of stress and the

duration of its action, revealing a differentiating effect on the spectrum of abnormalities of male sporo- and gametogenesis (De Storme et al. 2014). Given the increased sensitivity of male gametes to external factors, pollen analysis has been used to assess and identify resistant genotypes. Methods have been developed to determine genotype-specific responses to factors such as high, low temperatures and drought (Pressman 2002; Patil et al., 2006; Dolferus et al., 2013; Demurin et al., 2022), soil salinity (Gul et al., 2008; David Monica, 2012), plant disease damage (Chikkodi et al., 2000; Saltanovici, 2016) and even plant introduction (De Storme et al., 2014) in different crops based on pollen quality.

Positive results have been obtained in studying the mechanisms of adaptation of genotypes of various agricultural crops to the impact of anthropogenic factors – herbicide application, high ozone and carbon dioxide content in the air, acidity level of precipitation, accumulation of heavy metals in the soil, etc. (Bessonova et al., 1997; Roschina et al., 2001; Aloni B. et al., 2001; Pressman et al., 2002; Klimenko et al., 2005; Cratao et al., 2008). A relationship has been established between the resistance of pollen and sporophyte to changes in ambient temperature, the competitiveness of pollen and the growth rate of the sporophyte, characterizing the adaptability of plants (Mulcahy, 1974; Zamir & Cadish, 1987; Frova et al., 1995). The increased stability of genotypes as a result of selection based on the characteristics of the male gametophyte is explained by the partial expression of the same genes at the haploid and diploid phases of the plant life cycle (Sary Gorla et al., 1986; Larkindale & Vierling 2008; Frank et al., 2014). The gametophytic generation in the plant life cycle not only ensures gene transfer but also probably participates in the adaptive process.

Despite such a wide range of scientific developments devoted to the problems of the influence of unfavorable environmental factors on the formation and functioning of male gametophytes, the results of these studies are not widely used in practical breeding. The lack of methods for assessing the variability of various quantitative traits of the male gametophyte, which allows the judgement about the adaptive reaction and stability of genetic systems of certain genotypes to the action of abiotic environmental factors, limits the possibilities of pollen breeding. There is practically no information in scientific publications on the nature of the manifestation of male gametophyte traits under the influence of certain stress factors in first-generation hybrids, depending on the characteristics of their parental forms. At the same time, the questions related to the role of parental components in providing high adaptability to hybrid progeny in breeding for heterosis are of paramount importance. In this context, an attempt was made to study adaptive reactions and the degree of variability of morphological traits of the nuclei of vegetative and generative pollen cells in lines contrasting instability, including heterozygous progenies developed through hybridization with their participation. Knowledge of the character of manifestation and degree of variability of traits in heterozygous organisms depending on the stability of trait manifestation in their parental forms under the influence of temperature stress factors will allow the breeder to perform a subsequent purposeful and effective selection of parental components for crosses and obtain  $F_1$  hybrids with higher adaptive potential. The research aimed to study the variability of three quantitative morphological features of the nuclei of vegetative (**V**) and generative (**G**) tomato pollen cells (diameter, perimeter, area) in parental forms  $(P_1$  and  $P_2$ , homozygous lines) and their  $F_1$  hybrids (heterozygous populations of gametes) under the influence of high (45 ºС) and low temperature  $(+7 \degree C)$  stress, which will probably allow this information to be not potential but widely available for the development of new selection methods at the gametophyte level and the optimization of parental component selection to produce stable heterotic  $F_1$  hybrids.

## **2. Material and Methods**

#### *2.1. Experimental Material*

Heterotic F<sup>1</sup> tomato hybrids - Irok, Krasnaya Strela, Severny express, and their parental lines – L 187/1, L 187/2, L 214, L 828 and L 965 (author-creator Doctor of Agricultural Sciences, Professor S.I. Ignatova) were used as objects of research. The experimental sample plants were grown in summer greenhouses according to the methods generally accepted for tomato culture in the ecological zone of the Republic of Moldova.

#### *2.2. Object of research*

Male gametophyte (pollen) of tomato. The nuclei of vegetative (V) and generative (G) cells of pollen of  $F_1$  hybrids and their parental forms  $(P_1 \text{ and } P_2)$  were studied using the automated complex, "Morfokvant", consisting of a scanning light microscope, a processor that controls it, and a computer. The presence of a scanning probe and two measuring channels with a focal mirror device (FMD) makes it possible to determine the optical density of cells, cellular structures and their morphological particularities. The morphological traits of the nuclei of vegetative and generative cells of tomato pollen (diameter, perimeter, area) and the particular features of their variability under the action of high (45 ºС) and low (+7 ºС) temperatures were studied on permanent cytological preparations, which were made in three replicates.

#### *2.3. Production of permanent preparation*

Pollen for the study was isolated from flowers of  $F_1$ hybrid plants and their parental lines. Freshly collected pollen of each genotype was divided into three portions. One portion of the pollen (control) was fixed in Carnoy's solution (3:1), stained according to Schiff, and then permanent preparations were made according to Kravchenko's method (1986). The other two portions of the pollen were applied on glass slides in a monolayer and placed in thermostats at temperatures of 45 ºС for 6 hours and +7 ºС for 24 hours, respectively. After temporary exposure of the pollen to temperature stress factors,

it was fixed, and permanent preparations were made according to the methods described above. The diameter, perimeter and area of nuclei (relative units) of vegetative (V) and generative (G) tomato pollen cells of parental forms (P1 and P2) and their  $F_1$  hybrids in control (25 °C) and under the influence of high (45 °C) and low temperatures (+7 °C) was studied by counting and analyzing 100 nuclei for each cell and in each research treatment (control and experiments).

#### *2.4. Statistical Analysis*

The data obtained were processed in a dispersion complex using the Statistica 7 computer program, including cluster analysis, which provides the classification of genotypes based on the similarity and differences in the parameters of the studied traits, the diameter, perimeter, and area of the nuclei of vegetative (V) and generative (G) pollen cells. In the cluster dendrite, genotypes are divided into three clusters according to the level of similarity (Figures 1 and 2). This allows the identification of the population heterogeneity degree by the reaction of pollen of parental lines and the  $F_1$  hybrids produced on their basis to the action of temperature stress factors (Nandini B. et al).

#### **3. Results and Discussion**

Analysis of the pollen response in  $F_1$  hybrids and their parental forms to stress temperatures (45 °C and  $+7$  °C), taking into account the variability of the morphological traits, the diameter, perimeter and area of the nuclei of V and G cells of tomato pollen grains showed significant differences both between parental forms used in different combinations of crosses, and between the  $F_1$  hybrids derived on their basis. The nuclei of V and G pollen cells have been found to show different responses to the action of high and low temperatures. The root cause of their ambiguous response may be the structure, as the nuclei of V cells are vacuolated and large, in contrast to the nuclei of G cells, which are denser and smaller (Pausheva, 1986). It has been found that the nuclei of vegetative V cells in the populations of all  $F_1$  hybrids are morphologically more homogeneous than in their parental forms. According to the pattern of the manifestation of morphological traits of generative G cell nuclei, especially under the influence of stress temperatures, a higher variability was observed in the populations of  $F_1$  hybrids, which indicates a broad relationship between genomic changes associated with hybridization. In contrast, the level of changes in different hybrid combinations depends on the characteristics of their parental forms (Table 1). Perhaps this is also due to the fact that the nuclei of V and G pollen cells in homozygous and heterozygous genotypes differ in the composition of hereditary factors (Kravchenko, 1993).

## *3.1. Variability of Morphological Traits of Nuclei of Vegetative (V) Cells of Tomato Pollen Under Stress Temperatures*

An individual analysis of the response of the nuclei of vegetative V pollen cells of the parental forms and their  $F_1$  hybrids to high-temperature treatment revealed a decrease in the index of the trait nucleus area. These differences (25  $\rm{°C}$  / 45 °C) are significant for all three  $F_1$  hybrids. They are more pronounced in both parental forms of the  $F_1$  hybrid Irok and the maternal line  $F_1$  Severny express, less in the Krasnaya Strela hybrid (Table 1). The nuclei of V pollen cells react differently to their exposure to a low positive temperature  $(+7)$ °C). The differences are significant for the "nucleus diameter" in the maternal form and the Irok hybrid. Consistently high rates, relative to the control  $(25 \degree C)$ , as well as against a background with a high temperature, were detected in the Krasnaya Strela hybrid and its parental forms. The differences in the nucleus perimeter trait are highly significant only in the Severny express hybrid and its maternal form. Pollen of all genotypes also reacts to low temperatures with a decrease in the index of the nucleus area trait (Table 1), but differences (25 °C / 7 °C) are significant only in the Severny express hybrid and the  $F_1$  Irok combination. The nuclei of vegetative V cells of pollen grains are the most homogeneous after both high and low-temperature treatment in the  $F_1$  hybrid Krasnaya Strela and its parental forms (Table 1), which are characterized by equally high indices of resistance of their pollen to both stress factors under study. Clustering of the genotypes according to the pattern of the manifestation of morphological traits of the nuclei of V pollen cells at 25 ºС (control) revealed the following distribution: the first cluster included genotypes with average values of the traits (diameter, perimeter, nucleus area), the second cluster included genotypes with low values and the third with high values. When pollen is exposed to a temperature of 45 ºС, the degree of similarity between genotypes in populations changes, and they end up in different clusters, which indicates the presence of genotypes with ambiguous responses of vegetative pollen cell nuclei that are (more or less) resistant in populations and the possible use of indices for these traits to identify and select resistant genotypes. Whereas a temperature of 7 °C does not have a special effect on the variability of the traits of V cell nuclei, the degree of similarity between genotypes relative to the control remains almost unchanged (Fig. 1). It can be assumed that the investigated genotypes are more resistant to low temperature. In general, the pattern of the morphological trait manifestation of the V pollen cell nuclei in  $F_1$  is mainly determined by the influence of the paternal forms.

## *3.2. Variability of Morphological Traits of Nuclei of Generative (G) Cells of Tomato Pollen Under Stress Temperatures*

Analysis of the morphological traits (diameter, perimeter and area) of generative G pollen cell nuclei shows a more pronounced response to the action of high and lowtemperature stress factors (45 °C and 7 °C) relative to control (25 °C). The predominant part of the genotypes responded to stressful temperatures by reducing the trait indices, while in the maternal line of the Krasnaya Strela hybrid, the indices of all traits are higher against both stress backgrounds than in the control (Table 1). A high response of the generative cell nuclei

to the action of both stressful temperature factors is observed for the traits of nucleus diameter and perimeter, while the differences are more pronounced for the trait nucleus area against the background of high temperature and less pronounced against low temperature (Table 1). As a result, a higher variability of the morphological traits of G pollen cell nuclei was found at all backgrounds (25 ºС, 45 ºС, 7 ºС). Clustering of genotypes showed that, for example, in the control (25 ºС), the value and pattern of the manifestation of all traits is determined by the influence of the paternal component in all  $F_1$  hybrids, while a high influence of maternal forms is found in the treatments with temperature stress factors (45 ºС and 7 ºС) (Figure 2). Possible, resistance to temperature stresses is determined by maternal forms. In general, the response of the initial forms and hybrids of  $F_1$ tomato to the effect of temperature stresses for the pattern of the trait manifestation of G cell nuclei is more differentiated than that of the V cell nuclei. Simultaneously, genotypes in populations from different combinations of G cell nuclei against both backgrounds (45 ºС and 7 ºС) had a similar distribution into clusters (Figure 2). Probably, breeding for resistance to one factor can result in an increase in tolerance for another.

**Table 1. Morphological characteristics of the nuclei of vegetative (V) and generative (G) tomato pollen cells in parental lines and their F1 hybrids and the significance of differences in traits when exposed to high and low temperatures relative to the control**

	Nuclei of V pollen cell			Nuclei of G pollen cell							
<b>Genotypes</b>	<b>Diameter</b>	Perimeter	Area	<b>Diameter</b>	Perimeter	Area					
	25 °C (control)										
$P_1$	$4.7 \pm 0.06$	$15.2 \pm 0.19$	$12.6 \pm 0.27$	$5.93 \pm 0.18$	$16.57 \pm 0.30$	$9.32 \pm 0.47$					
P <sub>2</sub>	$5.2 \pm 0.08$	$16.3 \pm 0.23$	$14.3 \pm 0.30$	$5.68 \pm 0.19$	$15.41 \pm 0.41$	$9.56 \pm 0.36$					
$F_1$ Irok	$5.4 \pm 0.07$	$16.4 \pm 0.20$	$14.8 \pm 0.34$	$5.71 \pm 0.18$	$15.80 \pm 0.35$	$9.64 \pm 0.43$					
$P_1$ $\mathbf{P}_2$ F <sub>1</sub> Krasnaya strela	$5.8 \pm 0.11$	$18.1 \pm 0.31$	$16.2 \pm 0.24$	$5.14 \pm 0.11$	15.86±0.28	$9.08 \pm 0.28$					
	$5.3 \pm 0.12$	$17.3 \pm 0.29$	$16.9 \pm 0.41$	$5.63 \pm 0.10$	$20.41 \pm 0.44$	$16.81 \pm 0.34$					
	$5.4 \pm 0.09$	$17.1 \pm 0.33$	$17.0 \pm 0.35$	$6.15 \pm 0.17$	$17.93 \pm 0.27$	$15.70 \pm 0.33$					
$P_1$ P <sub>2</sub> $F_1$ Severny express	$5.5 \pm 0.10$	$17.2 \pm 0.27$	$16.3 \pm 0.37$	$4.90 \pm 0.11$	$12.70 \pm 0.31$ $8.80 \pm 0.34$						
	$4.9 \pm 0.09$	$15.9 \pm 0.34$	$14.4 \pm 0.40$	$5.51 \pm 0.15$	$14.56 \pm 0.22$	$11.52 \pm 0.26$					
	$5.4 \pm 0.11$	$16.3 \pm 0.36$	$15.9 \pm 0.36$	$5.65 \pm 0.19$	$15.48 \pm 0.33$	$12.33 \pm 0.37$					
	Heat temperature $(45^{\circ}C)$										
$P_1$	$4.9 \pm 0.07$	$16.1 \pm 0.14$ *	$11.2 \pm 0.22$ ***	$5.51 \pm 0.15$	$15.66 \pm 0.24$ *	$8.63 \pm 0.32$ **					
P <sub>2</sub> $F_1$ Irok	$4.3 \pm 0.06$	$16.3 \pm 0.17$	$12.5 \pm 0.16$ ***	$4.93 \pm 0.16$ ***	13.43±0.26 ***	$9.04 \pm 0.24$ *					
	$4.9 + 0.07$	$16.3 \pm 0.13$	$12.4 \pm 0.26$ ***	$5.07 \pm 0.16$ **	$14.11 \pm 0.33$ ***	$8.22 \pm 0.27$ **					
$\mathbf{P}_1$	$6.1 \pm 0.04$	$19.1 \pm 0.11$ **	$16.2 \pm 0.31$	$5.72 \pm 0.14$ *	$17.22 \pm 0.34$ ***	$10.14 \pm 0.41$ *					
P <sub>2</sub>	$5.7 \pm 0.18$	$17.9 \pm 0.20$	$16.6 \pm 0.26$	$5.56 \pm 0.16$	$19.80 \pm 0.48$ *	$16.16 \pm 0.50$					
$F_1$ Krasnaya strela	$6.0 \pm 0.06*$	$18.7 \pm 0.26$ **	$16.6 \pm 0.19$	$6.14 \pm 0.19$	$17.84 \pm 0.32$	13.85±0.38 ***					
$P_1$ P <sub>2</sub> $F_1$ Severny express	$5.3 \pm 0.05$	$17.1 \pm 0.14$	$14.6 \pm 0.24$ ***	$5.27 \pm 0.21$	$11.10\pm0.24$ *** $8.65 \pm 0.32$						
	$5.0 \pm 0.09$	$16.0 \pm 0.10$	$13.8 \pm 0.21$	$4.05 \pm 0.11$ ***	$14.42 \pm 0.19$	$10.12 \pm 0.23$ ***					
	$5.5 \pm 0.04$	$17.3 \pm 0.19$ **	$14.0 \pm 0.17***$	$4.82 \pm 0.17$ ***	$12.81 \pm 0.28$ *** $9.31 \pm 0.29$ ***						
	Low temperature $(7^{\circ}C)$										
$P_1$	$3.8 \pm 0.06*$	$15.3 \pm 0.10$	$12.0 \pm 0.25$ *	$5.27 \pm 0.21$ *	12.98±0.30 ***	$8.33 \pm 0.24$ **					
P <sub>2</sub>	$5.0 \pm 0.09$	$16.0 \pm 0.13$	$13.7 \pm 0.23$ **	$5.72 \pm 0.17$	$14.11 \pm 0.36$ **	$9.07 \pm 0.32$					
$F_1$ Irok	$4.6 \pm 0.05$ *	$16.1 \pm 0.13$	$14.6 \pm 0.19$	$5.21 \pm 0.14$ *	$13.05 \pm 0.28$ ***	$8.61 \pm 0.34$ ***					
$P_1$	$5.9 \pm 0.07$	$18.6 \pm 0.22$	$16.4 \pm 0.29$	$6.00 \pm 0.15$ **	$16.42 \pm 0.24$	$9.26 \pm 0.41$					
P <sub>2</sub> F <sub>1</sub> Krasnaya strela	$5.7 \pm 0.05$	$17.4 \pm 0.18$	$15.8 \pm 0.18$	$6.16 \pm 0.18$ *	$19.24 \pm 0.32$ *	13.04±0.37 ***					
	$5.8 \pm 0.09$	$17.6 \pm 0.15$	$16.1 \pm 0.24$ *	$6.04 \pm 0.16$	$16.33 \pm 0.36$ *	14.85±0.44 **					
$P_1$	$5.1 \pm 0.04$ *	$16.3 \pm 0.21$ **	$14.2 \pm 0.16$ ***	$4.29 \pm 0.11$ **	$10.81 \pm 0.28$ ***	$8.07{\pm}0.25$ *					
P <sub>2</sub>	$5.4 \pm 0.07$	$15.4 \pm 0.14$ *	$12.8 \pm 0.11$ ***	$5.08 \pm 0.19$	$13.13 \pm 0.17$ **	$9.81 \pm 0.34$ ***					
$F_1$ Severny express	$5.0 \pm 0.05$	$15.1 \pm 0.19$ **	$13.7 \pm 0.23$ ***	$4.84 \pm 0.13$ ***	$12.64 \pm 0.26$ ***	$10.12 \pm 0.38$ ***					
Note::	* - significant at 0.05% level; ** - by 0.01%; *** - by 0.001%, relative to 25 °C (control)										



**Fig. 1 Dendrogram of the similarity of parental forms and their F<sup>1</sup> hybrids of tomato according to the morphological features of the nuclei of vegetative V pollen cells freshly collected (25 °C) and pollen treated with high (45 °C) and low (7 °C) temperatures: 1. L 187/1, 2. L 965, 3. F<sup>1</sup> Irok; 4. L 187/2, 5. L 828, 6. F<sup>1</sup> Krasnaya strela; 7. L 965, 8. L 214, 9. F<sup>1</sup> Severny express**



 **Fig. <sup>2</sup> Dendrogram of the similarity of parental forms and their F<sup>1</sup> hybrids of tomato according to the morphological features of the nuclei of generative G pollen cells freshly collected (25 °C) and pollen treated with high (45 °C) and low (7 °C) temperatures: 1. L 187/1, 2. L 965, 3. F<sup>1</sup> Irok; 4. L 187/2, 5. L 828, 6. F<sup>1</sup> Krasnaya strela; 7. L 965, 8. L 214, 9. F<sup>1</sup> Severny express**

## *3.3. Cluster Dispersion by Morphological Traits of Tomato V and G Pollen Cells Nuclei (Control Experiments)*

Cluster analysis of parental forms and  $F_1$  hybrids using the *k-mean* centroid method showed that intercluster dispersion was higher than intracluster one for the morphological traits of the diameter, perimeter and area of the V pollen cell. nuclei in the control  $(25 \degree C)$  and in the treatments with high (45  $^{\circ}$ C) and low (7  $^{\circ}$ C) temperatures (Table 2). This attests to the successful differentiation and classification of genotypes into three clusters with the corresponding level of trait values - low, medium, and high.

Cluster analysis of variance for the pattern of the morphological trait manifestation of G pollen cell nuclei against three different backgrounds provided similar results with the exception of the nucleus diameter trait at 25 °C and 45 °C (Table 3).

The results of the analysis of variance (Tables 2 and 3) confirm significant differences between the clusters for all the traits of vegetative (V) and generative (G) pollen cell nuclei in parental forms and their  $F_1$  hybrids when exposed to high (45) <sup>o</sup>C) and low temperature (+7 °C) factors.

**Table 2. Cluster dispersion by morphological traits of V cell nuclei of freshly collected pollen and pollen treated with high and low temperatures**

Traits of vegetative $(V)$ pollen cell nuclei	<b>Intercluster</b> dispersion	df	<b>Intracluster</b> dispersion	df	F	P				
$25^{\circ}$ C (control)										
Diameter	0.5989	2	0.3300	6	5.4444	0.0448				
Perimeter	5.9383	2	0.9817	6	18.1477	0.0029				
Area	16.2414	2	3.7742	6	12.9099	0.0067				
Heat temperature $(45^{\circ}C)$										
Diameter	2.4539	2	0.4950	6	14.8720	0.0047				
Perimeter	8.2347	2	0.7942	6	31.1071	0.0007				
Area	15.5872	2	4.3550	6	10.7380	0.0104				
Low temperature $(7^{\circ}C)$										
Diameter	16.2414	2	3.7742	6	12.9190	0.0067				
Perimeter	15.5872	$\overline{2}$	4.3550	6	10.7375	0.0104				
Area	9.9808	2	2.6392	6	11.3454	0.0092				

**Table 3. Cluster dispersion by morphological traits of G cell nuclei of freshly collected pollen and pollen treated with high and low temperatures**



## **4. Discussions**

The study of the variability of morphological traits of the nuclei of V and G cells of freshly collected pollen and pollen exposed to high and low temperatures in parental forms and  $F_1$  hybrids created on their basis showed that their nuclei react differently to the action of these factors. The variability of the indices of the traits of V nuclei cells of pollen in parental homozygous lines is higher in the variants of heat-treated pollen than in  $F_1$  hybrids. In the nuclei of G cells, high variability in all traits is noted in heterozygous progeny  $(F_1)$ hybrids) both in relation to their parental forms and the action of stress factors. Perhaps this is due to the influence of different hereditary factors combined in one genome as a result of hybridization, or the reason may be the existence of a large asynchrony of the processes occurring during the formation, development and functioning of microgametophytes. Comparative individual analysis of variability of morphological traits of tomato V and G pollen nuclei in parental lines and  $F_1$  hybrids revealed genotypespecific differences both between the studied hybrids and within one hybrid combination between paternal and maternal forms. In the Severny express and Irok hybrids, the parental

forms differ in different degrees of variability (high, low) of morphological traits of V and G pollen cell nuclei under the influence of high and low temperatures. Meanwhile, in the  $F_1$ hybrids in all variants of the studies, the trait indices are at the level of the parent forms with lower values (Table 1). Different results were obtained for the  $F_1$  Krasnaya Strela hybrid, in which the morphological trait indices of V and G pollen cell nuclei are at the level of the parental form with high values or average parental. Both parents of the Krasnaya Strela hybrid are distinguished by a stable manifestation of the studied traits of the nuclei of V and G cells, both freshly collected and heat-treated pollen (Table 1). The degree of variability of morphological traits of nuclei G cells of pollen (control experiments) in the  $F_1$  hybrid Krasnaya Strela is the lowest, which indicates its high adaptive potential relative to the tested high- and low-temperature stress factors. According to the author-creator (S. Ignatov), the Krasnaya Strela hybrid is characterized by a high heterotic effect for a large number of economically valuable traits; it is highly productive and is able to set fruits in the conditions of low temperatures and low light. Whereas the other two  $F_1$  hybrids, Irok and Severny express, have a less pronounced heterotic effect and for a lower number of traits. In general, it should be noted that the response of both V and G cell nuclei of pollen to low temperature was less pronounced than to high temperature. A particularly high degree of variability in the indices of morphological traits is observed in the nuclei of G cells of pollen after high-temperature exposure. It can be assumed that the studied  $F_1$  hybrids and their parental lines are characterized by high resistance to low temperatures and are more sensitive to the action of high-temperature stress. The study of processes occurring at the reproductive stages of plant development under the influence of unfavorable environmental factors, taking into account the variability of traits directly or indirectly affecting their productivity, will allow us to develop methods for assessing and selecting economically valuable genotypes.

#### **5. Conclusion**

 It has been found that the nuclei of vegetative V cells in the populations of all  $F_1$  hybrids are morphologically more homogeneous than those of their parental forms. On the contrary, the variability is higher in  $F_1$  hybrid populations for the traits of generative G cell nuclei, especially under the influence of temperature stress factors (45  $\degree$ C and +7  $\degree$ C), which indicates a broad relationship between genomic changes associated with hybridization. Genotypic differences are shown both between the  $F_1$  hybrids studied and within the same hybrid combination between paternal and maternal forms in regard to the pattern of the manifestation of morphological traits under the influence of temperature stresses. It has been established that in combinations where the maternal form is characterized by consistently high indices of G pollen cell nuclei, these indices are also higher in  $F_1$  hybrids.

This is especially pronounced against backgrounds with temperature stress factors. A hybrid combination  $(F_1 Krasnava)$ Strela) with a high and stable manifestation of the V and G pollen cells traits under the influence of stress temperatures has been identified, which indicates its high adaptive potential. Presumably, it is feasible to assess and select the source material and correctly plan crossings to produce hybrids with more stable genomes and high adaptability through the prism of changes in the indices of morphological traits of vegetative (V) and generative (G) pollen cells nuclei in parental forms and their  $F_1$  hybrids under the influence of high (45 °C) and low temperature (+7 ºС) stress factors.

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#### **References**

- [1] Beny Aloni et al., "The Effect of High Temperature and High Atmospheric CO<sup>2</sup> on Carbohydrate Changes in Bell Pepper (*Capsicum Annuum*) Pollen in Relation to its Germination," *Physiologia Plantarum*, vol. 112, no. 4, pp. 505-512, 2001. [\[CrossRef\]](https://doi.org/10.1034/j.1399-3054.2001.1120407.x) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=The+Effect+of+High+Temperature+and+High+Atmospheric+CO2+on+Carbohydrate+Changes+in+Bell+Pepper+%28Capsicum+annuum%29+Pollen+in+Relation+to+its+Germination&btnG=) [\[Publisher Link\]](https://onlinelibrary.wiley.com/doi/abs/10.1034/j.1399-3054.2001.1120407.x)
- [2] S.B. Chikkodi, and R.L. Ravikumar, "Influence of Pollen Selection for Alternaria Helianthi Resistance on the Progeny Performance against Leaf Blight in Sunflower (*Helianthus Annuus* L.)," *Sexual Plant Reproduction*, vol. 12, pp. 222-226, 2000. [\[CrossRef\]](https://doi.org/10.1007/s004970050004) [\[Google](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Influence+of+Pollen+Selection+for+Alternaria+Helianthi+Resistance+on+the+Progeny+Performance+Against+Leaf+Blight+in+Sunflower+%28Helianthus+annuus+L.%29&btnG=)  [Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Influence+of+Pollen+Selection+for+Alternaria+Helianthi+Resistance+on+the+Progeny+Performance+Against+Leaf+Blight+in+Sunflower+%28Helianthus+annuus+L.%29&btnG=) [\[Publisher Link\]](https://link.springer.com/article/10.1007/s004970050004)
- [3] P.L. Cratao et al., "Acquired Tolerance of Tomato (*Lycopersicon Esculentum*. CV. Micro-Tom) Plants to Cadmium Induced Stress," *Annals Applied Biology*, vol. 153, no. 3, pp. 321-333, 2008. [\[CrossRef\]](https://doi.org/10.1111/j.1744-7348.2008.00299.x) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Acquired+Tolerance+of+Tomato+%28Lycopersicon+esculentum.+CV.+MicroTom%29+Plants+to+Cadmium+Indened+Stress&btnG=) [\[Publisher Link\]](https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1744-7348.2008.00299.x)
- [4] Monica David, "Pollen Grain Expression of Intrinsic and Osmolite Induct Osmotic Adjustment in a Set of Heat Cultivars," *Romaine Agricultural Research*, no. 29, pp. 45-52, 2012. [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Pollen+Grain+Expression+of+Intrinsic+and+Osmolite+Induct+Osmotic+Adjustment+in+a+Set+of+Heat+Cultivars&btnG=)
- [5] Nico De Storme, and Danny Geelen, "The Impact of Environmental Stress on Male Reproductive Development in Plants: Biological Processes and Molecular Mechanisms," *Plant Cell and Environment*, vol. 37, no. 1, pp. 1-18, 2014. [\[CrossRef\]](https://doi.org/10.1111/pce.12142) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=The+Impact+of+Environmental+Stress+on+Male+Reproductive+Development+in+Plants%3A+Biological+Processes+and+Molecular+Mechanisms&btnG=) [\[Publisher Link\]](https://onlinelibrary.wiley.com/doi/full/10.1111/pce.12142)
- [6] Ya.N. Demurin, and O.A. Rubanova, "Pollen Plant Analysis of Different Sunflower Genotypes" *Oilseed Crops*, vol. 2, no. 186, pp. 10- 17, 2021. [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Pollen+Plant+Analysis+of+Different+Sunflower+Genotypes&btnG=)
- [7] Rudi Dolferus et al., "The Physiology of Reproductive-Stage Abiotic Stress Tolerance in Cereals," *Molecular Stress Physiology of Plants*, pp. 193-216, 2013. [\[CrossRef\]](https://doi.org/10.1007/978-81-322-0807-5_8) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=The+Physiology+of+Reproductive-Stage+Abiotic+Stress+Tolerance+in+Cereals&btnG=) [\[Publisher Link\]](https://link.springer.com/chapter/10.1007/978-81-322-0807-5_8)
- [8] Margaret H. Frank, and Micael J. Scanlon, ["Transcriptomic Evidence for the Evolution of Shoot Meristem Function in Sporophyte-](https://pubmed.ncbi.nlm.nih.gov/26416978/)[Dominant Land Plants through Concerted Selection of Ancestral Gametophytic and Sporophytic Genetic Programs,"](https://pubmed.ncbi.nlm.nih.gov/26416978/) *Molecular Biology and Evolution*, vol. 32, no. 2, pp. 355-367, 2014. [\[CrossRef\]](https://doi.org/10.1093/molbev/msu303) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Transcriptomic+Evidence+for+the+Evolution+of+Shoot+Meristem+Function+in+Sporophyte-Dominant+Land+Plants+through+Concerted+Selection+of+Ancestral+Gametophytic+and+Sporophytic+Genetic+Programs&btnG=) [\[Publisher Link\]](https://academic.oup.com/mbe/article/32/2/355/1052231)
- [9] C. Frova et al., "Sporophytic and Gametophytic Components of Thermotolerance Effected by Pollen Selection," *Journal of Heredity,* vol. 86, no. 1, pp. 50-54. 1995. [\[CrossRef\]](https://doi.org/10.1093/oxfordjournals.jhered.a111525) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Sporophytic+and+Gametophytic+Components+of+Thermotolerance+Effected+by+Pollen+Selection&btnG=) [\[Publisher Link\]](https://academic.oup.com/jhered/article-abstract/86/1/50/802959)
- [10] Humaira Gul, and Rafiq Ahmad, "Effect of Salinity on Pollen Viability of Different Canola *(Brassica napus L.)* Cultivars as Reflected by the Formation of Fruits and Seeds," *Pakistan Journal of Botany*, vol. 38, no. 2, pp. 237-247, 2006. [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Effect+of+Salinity+on+Pollen+Viability+of+Different+Canola+%28Brassica+napus+L.%29+Cultivars+as+Reflected+by+the+Formation+of+Fruits+and+Sids&btnG=)
- [11] Anatoly Nikolaevich Кravchenko, Features of the Formation of Genetic Variability in the Process of Sporogametophyto- and Embryogenesis in Plants. [Online]. Available: [https://earthpapers.net/osobennosti-formirovaniya-geneticheskoy-izmenchivosti-v](https://earthpapers.net/osobennosti-formirovaniya-geneticheskoy-izmenchivosti-v-protsesse-sporogametofito-i-embriogeneza-u-rasteniy)[protsesse-sporogametofito-i-embriogeneza-u-rasteniy](https://earthpapers.net/osobennosti-formirovaniya-geneticheskoy-izmenchivosti-v-protsesse-sporogametofito-i-embriogeneza-u-rasteniy)
- [12] Jane Larkindale, and Elizabeth Vierling, "Core Genome Responses Involved in Acclimation to High Temperature," *Plant Physiology*, vol. 146, no. 2, pp. 748-761, 2008. [\[CrossRef\]](https://doi.org/10.1104%2Fpp.107.112060) [\[Google Scholar\]](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2245833/) [\[Publisher Link\]](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2245833/)
- [13] Anida Mesihovic et al., "Heat Stress Regimes for the Investigation of Pollen Thermotolerance in Crop Plants," *Plant Reproduction*, vol. 29, pp. 93–105, 2016. [\[CrossRef\]](https://doi.org/10.1007/s00497-016-0281-y) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Heat+Stress+Regimes+for+the+Investigation+of+Pollen+Thermotolerance+in+Crop+Plants&btnG=) [\[Publisher Link\]](https://link.springer.com/article/10.1007/s00497-016-0281-y)
- [14] David L. Mulcahy, "A Correlation between Gametophytic and Sporophytic Characteristics in Zea *Mays* L.," *Science,* vol. 171, no. 3976, pp. 1155-1156, 1971. [\[CrossRef\]](https://doi.org/10.1126/science.171.3976.1155) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=A+Correlation+Between+Gametophytic+and+Sporophytic+Characteristics+in+Zea&btnG=) [\[Publisher Link\]](https://www.science.org/doi/abs/10.1126/science.171.3976.1155)
- [15] B. Nandini et al., "Genetic Variability Analysis for Grain Yield and its Components Traits in Traditional Rice Varieties," *International Journal of Current Microbiology and Applied Sciences*, vol. 6, no. 8, pp. 494-502, 2017. [\[CrossRef\]](https://doi.org/10.20546/ijcmas.2017.608.064) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Genetic+Variability+Analysis+for+Grain+Yield+and+its+Components+Traits+in+Traditional+Rice+Varieties&btnG=) [\[Publisher Link\]](https://www.ijcmas.com/abstractview.php?ID=3516&vol=6-8-2017&SNo=64)
- [16] Ettore Pacini, and Rudi Dolferus, "The Trails and Tribulations of the Plant Male Gametophyte- Understanding Reproductive Stage Stress Tolerance," *Abiotic and Biotic Stress in Plants*, 2016. [\[CrossRef\]](http://dx.doi.org/10.5772/61671) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=The+Trials+and+Tribulations+of+the+Plant+Male+Gametophyte+%E2%80%94+Understanding+Reproductive+Stage+Stress+Tolerance&btnG=) [\[Publisher Link\]](https://www.intechopen.com/chapters/49604)
- [17] Z.P. Pausheva, *Workshop on Plant Cytology*, Moscow, Sprint-Nauka, pp. 1-271, 1988. [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=%D0%9F%D1%80%D0%B0%D0%BA%D1%82%D0%B8%D0%BA%D1%83%D0%BC+%D0%BF%D0%BE+%D1%86%D0%B8%D1%82%D0%BE%D0%BB%D0%BE%D0%B3%D0%B8%D0%B8+%D1%80%D0%B0%D1%81%D1%82%D0%B5%D0%BD%D0%B8%D0%B9+-+%D0%9F%D0%B0%D1%83%D1%88%D0%B5%D0%B2%D0%B0+%D0%97.%D0%9F.&btnG=) [\[Publisher Link\]](http://booksshare.net/index.php?id1=4&category=biol&author=pausheva-zp&book=1988)
- [18] Basavanagounda S. Patil, R.L. Ravicumar, and P.M. Salimath, "Effect of Pollen Selection for Moisture Stress Tolerance on Progeny Performance in Sorghum," *Journal of Food, Agriculture & Environment*, vol. 4, no. 1, pp. 201-204, 2006. [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Effect+of+Pollen+Selection+for+Moisture+Stress+Tolerance+on+Progeny+Performance+in+Sorghum&btnG=) [Publisher [Link\]](https://www.wflpublisher.com/Abstract/794)
- [19] P.L. Рfahler, "Comparative Effectiveness of Pollen Genotype Selection in Higher Plants," *Pollen: Biology and Implications for Plant Breeding*, pp. 361-366. 1982. [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Comparative+Effectiveness+of+Pollen+Genotype+Selection+in+Higher+Plants&btnG=) [\[Publisher Link\]](https://www.cabidigitallibrary.org/doi/full/10.5555/19851642158)
- [20] Etan Pressman, Mary M. Peet, and D. Mason Pharr, "The Effect of Heat Stress on Tomato Pollen Characteristics is Associated with Changes in Carbohydrate Concentration in the Developing Anthers," *Annals of Botany*, vol. 90, no. 5, pp. 631-636, 2002. [\[CrossRef\]](https://doi.org/10.1093/aob/mcf240) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=The+Effect+of+Heat+Stress+on+Tomato+Pollen+Characteristics+is+Associated+with+Changes+in+Carbohydrate+Concentration+in+the+Developing+Anthers&btnG=) [\[Publisher Link\]](https://academic.oup.com/aob/article/90/5/631/205550)
- [21] V.V. Roschina, and E.V. Melnikova, "Chemosensitivity of Pollen to Ozone and Peroxides," Russian Journal of *Plant Physiology*, vol. 48, pp. 74-83, 2001. [\[CrossRef\]](https://doi.org/10.1023/A:1009054732411) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Chemosensitivity+of+Pollen+to+Ozone+and+Peroxides&btnG=) [\[Publisher Link\]](https://link.springer.com/article/10.1023/A:1009054732411)
- [22] M. Sari Gorla et al., "The Extent of Gametophytic-Sporophytic Gene Expression in Maize," *Theoretical and Applied Genetic*, vol. 72, pp. 42-47, 1986. [\[CrossRef\]](https://doi.org/10.1007/BF00261452) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=The+Extent+of+Gametophytic-Sporophytic+Gene+Expression+in+Maize&btnG=) [\[Publisher Link\]](https://link.springer.com/article/10.1007/BF00261452)
- [23] D. Zamir, and I. Gadish, "Pollen Selection for Low Temperature Adaptation in Tomato," *Theoretical and Applied Genetics*, vol. 74, pp. 545-548, 1987. [\[CrossRef\]](https://doi.org/10.1007/BF00288849) [\[Google Scholar\]](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Pollen+Selection+for+Low+Temperature+Adaptation+in+Tomato&btnG=) [\[Publisher Link\]](https://link.springer.com/article/10.1007/BF00288849)