THE ROLE OF MICROGAMETOPHYTE SELECTION FOR IDENTIFICATION OF TOMATO FORMS RESISTANT TO ABIOTIC STRESS FACTORS

ROLUL SELECȚIEI MICROGAMETOFIȚELOR ÎN IDENTIFICAREA FORMELOR DE TOMATE REZISTENTE LA FACTORII DE STRES ABIOTIC

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Milania MAKOVEI
ORCID: 0009-0009-5039-6270
E-mail: milania.makovei@sti.usm.md
Moldova State University, Chisinau, Republic of Moldova

Sumar. *Cercetările au fost efectuate într-una dintre cele mai vulnerabile etape ale ontogenezei plantei de tomate – stadiul de gametofit mascul matur. Sunt prezentate rezultatele unei evaluări complexe și ale unei analize comparative a probelor de colecție privind rezistența la temperaturi ridicate și scăzute, în funcție de stadiul de vegetație a plantelor. Este prezentată eficiența selecției etapizate prin manifestarea stabilă a caracterelor gametofitului mascul, pentru crearea materialului de bază cu rezistență complexă la diferiți factori de stres termic. Sunt selectate probe cu rezistență determinată genetic la temperaturi de stres ridicat și scăzut, pentru utilizare ulterioară în programele de ameliorare ca donatori pentru crearea de soiuri și hibrizi de tomate cu un nivel mai ridicat de rezistență la factorii de mediu nefavorabili.*

Cuvinte-cheie: *tomate, microgametofit, evaluare, selecție, factori de stres termic.*

INTRODUCTION

In recent years, significant results have been achieved in tomato breeding in creating varieties and hybrids with high productivity. However, global climate change and frequently recurring viral epiphytoties, including in the Republic of Moldova, lead to intensification of genetic erosion, and as a consequence, a decrease in the potential productivity and quality of the yield of existing tomato varieties and hybrids. This necessitates the creation of new forms capable of realizing the genetically embedded productivity potential in conditions other than optimal. This can be facilitated by an integrated approach to research using effective methods of evaluation, identification and creation of source material involving new germplasm with higher genetic divergence in the breeding process. Genetically and geographically distant forms, wild and semi-cultivated varieties, as well as mutant forms of tomato can be used as such. The possibility of involving a large number of genotypes in artificial selection [1, 2], regulating the "rigidity" of selection under controllable conditions [3], requires the development of more accurate and effective approaches to assessing and identifying valuable genotypes using pollen populations as a test system to determine their adap-

tive potential in rapidly changing environmental conditions throughout the entire vegetation period of plants [4]. Active integration of gamete technologies into traditional breeding programs will allow for a simultaneous, quick and effective study of the features of manifestation and variability of biological and economically valuable traits, both depending on the characteristics of the genotype and the action of environmental factors at different stages of plant development. This approach to research suggests the possibility of intensifying the selection process aimed at creating new varieties and heterotic hybrids of tomato with a complex set of economically valuable traits that combine high productivity and resistance to stressful abiotic, biotic and other environmental factors.

Based on this, the aim of this study is to develop breeding technologies that allow for a short period of time to evaluate and analyze a large amount of experimental material and to isolate valuable genotypes with a high potential for resistance to the effects of temperature stress factors at one of the most vulnerable stages of plant ontogenesis - the stage of mature male gametophyte.

MATERIALS AND METHODS

The experimental material consisted of 60 tomato samples, including carriers of mutant marker genes (*sp, sp*$^{+}$*, sp*$^{\pm}$*, rin, nor, alc, ls, br, u, hp-1, j-2, Ln, W*^{om}*, ps, ps-2* and others) that control the manifestation of a number of economically valuable traits. The plants for the studies were grown in the greenhouse of the experimental section of the Institute of Genetics, Physiology and Plant Protection, on 10 m^2 plots with 30 accounting plants from each genotype [5]. Phenological observations, morphological description and recording of quantitative indicators of the main breeding-valuable traits (type of inflorescence, number of flowers on plants, number of set fruits, total yield, marketability of fruits, resistance to main diseases, etc.) were carried out [6]. Evaluation of resistance to high and low temperatures was carried out based on a set of traits of the male gametophyte [3]. Genotypes with a high degree of coincidence of indicators of economically valuable traits were selected, including those controlled by mutant genes with resistance to high and low temperature conditions. Pollen for the studies was collected from flowers of plants of each genotype individually at three different times: in the phase of mass flowering of the first two inflorescences (June-July); in the phase of flowering of flowers on inflorescences of the middle tier of plants (July-August) and in the phase of mass fruiting and flowering of flowers of inflorescences of the upper tiers (August-September). Pollen was collected from fully opened flowers in the morning hours using a single method. At different stages of evaluation, freshly collected pollen from each genotype was divided into three parts. One was immediately sown on an artificial nutrient medium (15% sucrose and 0.006% boric acid) and germinated in vitro for 3 hours at 25° C. The pollen viability-germination (*p, %*) and pollen tube length (*l, μm*) were determined in ocular microm-

eter divisions; this served as a control (C). The second part was used to assess heat resistance by treating it at 45° C for 8 hours, followed by germination to determine viability (*p, %*) and pollen tube length (*l, μm*). The cold resistance of genotypes was diagnosed by sowing a third of the pollen on a nutrient medium, which was germinated in a cold thermostat (XT – 3/70) at +6°C for 24 hours, after which its viability (*p, %*) and tube length (*l, μm*) were also determined. For each genotype, at different stages and in each variant of the study, at least 500 pollen grains were assessed. All pollen preparations were examined under a Zeiss light microscope. The criterion for classifying a genotype into a certain resistance group is the resistance coefficient, which reflects the ratio between pollen viability and pollen tube length against the background of each of the stress factors used to the control

$$Kstr = (Pex \times Lex) : (Pc \times Lc) \times 100\%$$

where: *K* - is the stress resistance coefficient, expressed in %; Pex - is the number of germinated pollen grains in the experiment and *Lex* - is the length of pollen tubes in the experiment; *Pc* - is the number of germinated pollen grains in the control and *Lc* - is the length of pollen tubes in the control. Genetic-statistical analysis of the studied quantitative traits made it possible to determine the degree of genetic stability relative to each trait, individually for each genotype, depending on the characteristics of the genotype, the stage of plant development and the conditions of their cultivation.

RESULTS AND DISCUSSION

A comparative analysis of the results of assessing tomato collection samples for resistance to high and low temperatures by the nature of the manifestation of male gametophyte traits on different temperature stress backgrounds, including taking into account the stage of plant development, did not reveal a close correlation between them. The correlation coefficient between the level of heat resistance and cold resistance by male gametophyte traits both within one genotype and between them varied in a very wide range from -0.11 to +0.67.

For a deeper understanding of the value and significance of the results obtained, the studied genotypes were divided into appropriate groups according to the developed resistance scale (Table 1). Their ranking into groups is based on the nature of the manifestation of a complex of microgametophyte traits: germination of freshly collected pollen and the length of pollen tubes, resistance to germination and length of pollen tubes after exposure to high temperatures and the ability to germinate and form tubes of sufficient length for fertilization against a background of low temperatures (Table 1).

Table 1. Resistance scale for assessing the response of tomato genotypes to the action of stressful abiotic factors based on male gametophyte traits

No Croups	Traits		Characterization of genotype resistance
	Pollen resistance, %	Resistance by pollen tube length, %	
	Values of trait indicators within groups, %		
I	0 - 11	0 - 20	unresistance
II	12 - 23	21 - 41	low resistance
III	24 - 35	42 - 62	relatively resistance
IV	36 -47	63 - 83	resistant
V	48 - 59	84 - 104	highly resistant
VI	60 and >	105 and >	ultra resistant
VII	0 - 23	63 and >	low pollen resistance and > high resistance along the length of the pollen
VIII	48 and >	0 - 31	high pollen resistance and < low resistance along the length of the pollen tubes

Genetic determination of the pollen response to the action of artificially created stress temperature backgrounds in the studied tomato samples was established. This allowed them to differentiate and systematize them into appropriate groups, including depending on the stage of plant development. Genotypes with a lower response of their pollen to the action of both studied temperature stress factors were identified. They are distributed in the first group and are represented by 18 genotypes (Table 2). Their pollen practically does not react to the action of both temperature stress factors, and in some cases the values of the studied traits are higher, this is especially pronounced during germination against the background of a low-temperature stress factor. Along with these, genotypes (group 2) with high resistance of pollen obtained from flowers of inflorescences of lower tiers of plants (2-3 inflorescence) were identified, but as the level of the inflorescence position on the plant increases, the sensitivity of pollen to the effects of stressful temperature conditions increases. The third group included genotypes whose pollen, regardless of the level of the inflorescence position on the plant, demonstrates high resistance to the high-temperature stress factor, but at the same time the indicators sharply decrease against the background of low temperature. The decrease in the indicators of both traits (pollen germination and the ability of germinated pollen grains to form tubes of sufficient length for fertilization) is especially pronounced as the level of the inflorescence position on the vegetative plant from whose flowers the pollen was obtained increases (Table 2). The genotypes presented in the 4th and 5th groups are characterized by high intrapopulation variability of pollen both in morpho-biological characteristics of

pollen grains (different in shape, size and completeness) and in their ability to germinate and form pollen tubes of sufficient length for fertilization under the influence of stress factors. For example, in some cases, pollen germinates poorly, but the germinated pollen grains form long pollen tubes, while in other cases, pollen germinates actively, but the germinated grains form very short tubes with deformations of varying degrees of severity (Table 2). Such pollen characteristics are an obstacle to the successful fertilization process in natural conditions, which reduces the value of these forms for use in breeding programs as donors of resistance.

Table 2. Distribution of tomato collection samples into groups according to the nature of manifestation of male gametophyte traits under different stress conditions during plant vegetation

Ranking of genotypes into groups by type and degree of resistance	**Resistance group according to the scale depending on the stage of plant development**					
	To high temperature			**To low temperature**		
	Inflorescences					
	2 -3	**4 -5**	**and higher**	**2 - 3**	**4 - 5**	**and higher**
1. Mo 120, Mo 372, Mo 500, Mo 620, Mo 651, La2529, La3013, L79, L158, L 187, L234, L828, MaKrista, Fakel, Stefani, PetraMak, Matriona	VI	V	V	V	V	VI
2. Mo 56, Mo 308, La 3616, Uspeh, Potoc, Frigusor, Onix, L965, L111	IV	III	II	VII	IV	III
3. L28, L224, L386, L454, L456, L479, L828, Demidov, Viza, Solaris, Ballada, Barnaulskii conservnii, Vivat	VI	V	V	IV	III	II
4. L7, L8/234, L16, L33/241, L201, L423, L556, Mo 588, Mo 900	IV	IV	IV	I	I	I
5. L110/18, L118, L123, L135, L171, L186, L193	VIII	VIII	VIII	VII	VII	VII
6. L555, Mo 443, Mo 606, L45/16, L11/4, L11069	II	I	I	III	IV	IV

The obtained results show that modeling artificial stress backgrounds in laboratory conditions allows for the evaluation and selection of resistant tomato genotypes, including taking into account the variability of traits depending on the age of the plants (Figure 1 a, b). The analysis of the dynamics of change in the trait indices depending on the inflorescence position level on the vegetative plant confirmed the clearly expressed differences between the studied genotypes, including their response to increased temperature

conditions under natural conditions. As the inflorescence position level increases and temperature conditions increase during plant vegetation, pollen of the first group of genotypes retains high resistance (Figure 1 a, b). Stable manifestation of traits is observed in genotypes from groups 4, 5 and 6 when exposed to high temperature. Whereas, against the background of a low-temperature stress factor, pollen of genotypes from group 5 practically does not germinate, which indicates low or complete absence of resistance to the action of this stress factor. Significant variability in the indices of both pollen traits depending on the inflorescence position level on the vegetative plant in its response to low temperature is also observed in genotypes of groups 4 and 6 (Figure 1 a, b).

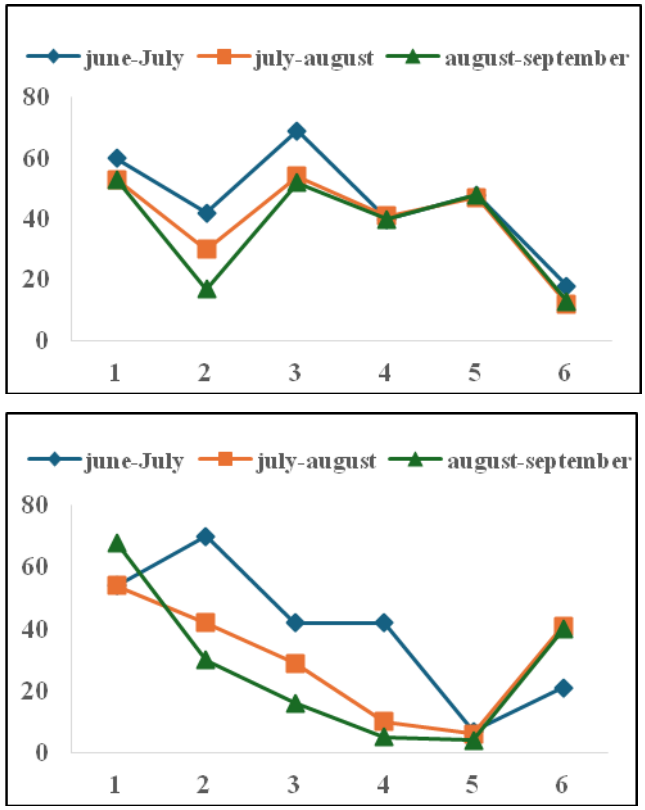


Figure 1 (a, b) Dynamics of changes in pollen resistance of tomato collection samples to high and low temperatures in different groups during the growing season

The pronounced differences between the collection samples on the action of two different temperature stress factors indicate that resistance to them is determined by different genetic systems. The revealed individual features of the genotypes, reflected in different spectra of variability of the studied traits both within one genotype and between them in relation to their reaction to the action of external factors during the vegetation

period of plants, confirm this conclusion. Such an approach to research allowed us to conduct an effective assessment and select valuable source material with a stable manifestation of male gametophyte traits throughout the vegetation period of plants under the action of two different stress temperature factors, which characterizes them as highly resistant.

CONCLUSION

In the process of step-by-step evaluation of pollen of tomato collection samples on the effect of high and low temperatures during the entire period of plant vegetation, it was shown that resistance to temperature stress factors in the microgametophyte is genetically determined. This made it possible to isolate the most valuable genotypes of both the cultural type and, from among the carriers of valuable mutant marker genes. Their subsequent use as donors in crossings will allow us to establish the role of resistance of the male gametophyte in the formation of reactions of hybrid offspring to the effect of stressful abiotic factors and their selection and genetic significance in solving practical selection problems.

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