POLLEN REACTION OF MUTANT TOMATO FORMS TO ABIOTIC STRESS FACTORS

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The article presents the results of screening accessions of the mutant tomato gene pool (125 genotypes) based on the response of their pollen to abiotic stress factors (high, low temperatures, drought) in order to study their potential and identify genetic sources/donors of resistance. The studies were carried out in open ground conditions from 2015 to 2017 and in summer greenhouses from 2017 to 2019. High heterogeneity of the genotype-specific response of pollen of mutant forms to various stress factors was shown: with a lethal outcome to all factors (10 genotypes); highly resistant to all stressors (20 genotypes); combining resistance: to high and low temperatures (7 genotypes), to high temperature and drought (9 genotypes), to cold and drought (4 genotypes); resistant only: to high (14 genotypes) or low (17 genotypes) temperatures, drought (6 genotypes). Mutant forms (38 genotypes) with high intrapopulation variability in pollen germination and the ability to form long pollen tubes under various stressors were also identified. It was established that resistance to heat, cold and drought according on traits of the male gametophyte was determined by different genetic systems.

Key words: tomato, mutant forms, traits, male gametophyte, resistance, stress factors, high temperature, low temperature, drought.

Abbreviations: divisions of the eyepiece micrometer (d.e.m.).

Introduction. Tomato is one of the main vegetable crops grown everywhere (about 5 million hectares) both in open and in protected ground [1]. Its high share in the gross production of vegetables is explained by high ecological plasticity, that is, the ability to grow and bear fruits in different climatic zones, high yields, multi-purpose use of fruits, their high biological value and rapid mechanization of the main processes during its cultivation. As a classical object, it is extensively used in genetic and molecular studies [2-6], contributing to progress in genetics and breeding. Breeders provide a lot of information to tomato genetics and, conversely, fundamental research into this crop contributes to significant breeding successes [3]. At the same time, it should be noted that the breeding efficiency is limited by limited genetic variability of the cultivated tomato gene pool available for selection. A growing deficit of the genetic diversity and, as a result, a decrease in the adaptability of current varieties and hybrids, their vulnerability to biotic, abiotic and anthropogenic stresses require more active involvement of new sources of germplasm with more pronounced genetic divergence in breeding. Of particular interest in this regard are mutations obtained by various methods, which are increasingly involved in breeding to genetically improve the existing varietal diversity and obtain new forms of tomatoes with original combinations of economically valuable traits [2, 7, 8, 9]. However, the potential of mutant tomato forms has not been studied at all in terms of their resistance to abiotic stresses and other factors. Availability of such information can contribute to their more extensive involvement in breeding to solve a wide and versatile range of problems in the process of creating complex gene carriers, varieties and hybrids capable of realizing the genetically inherent productivity potential under

stressful conditions. Consequently, the depth of knowledge of genotypic characteristics of mutant tomato forms regarding their responses to one or several unfavorable factor in the crop cultivation zone at different stages of ontogenesis largely determines the prediction of breeding results and effectiveness of their implementation.

Taking into account the importance of the reproductive system in the tomato development and formation of its productivity, we studied the genetic potential of mutant tomato forms regarding their resistance to high and low temperatures and drought at one of the most vulnerable stages of plant development – the mature male gametophyte stage.

In connection with climatic changes on the planet and, in particular, in Europe, the primary objectives of both fundamental and applied studies are to assess plant resistance to abiotic, biotic and other stress factors. The urgency of the problem of mobilizing plant resources is increasing, especially in regions that are most susceptible to climatic disasters, which lead to intensified genetic erosion and, as a consequence, to a significant reduction in the potential productivity of not only tomatoes, but also of other agricultural crops [10, 11, 12]. Various authors state that ecological sustainability in the plant world is one of the scarcest economically valuable traits [13, 14, 11, 12]. This necessitates a wider involvement of identified trait plant gene pools in breeding, which N.I. Vavilov [15] considered as the main source of primary starting material. However, until now the concept and principles of organizing such collections remain insufficiently developed, and this is observed not only in relation to local, but also to world collections. For example, among a million accessions collected in the world's collections of cultivated plants, less than 1% has been phenotypically characterized [16]. This indicates the need to study, identify, create and preserve identified trait genetic collections that would contribute to the creation of varietal diversity, with high ecological plasticity, capable of realizing the genetically inherent productivity potential in adverse environmental conditions. Collections of mutant genes are of particular interest in this regard. Some authors demonstrated the effectiveness of using mutant tomato forms in breeding and genetic studies [2, 3, 8, 17] as well as for solving practical breeding problems, on the basis of which a number of multi-marker highly productive varieties and lines of tomato were bred; subsequently, they were used to obtain high-tech heterotic hybrids F_1 [7, 9, 18]. However, collections of mutant forms have not been studied in terms of their resistance to abiotic stress factors. The complexity of solving this challenge lies in the fact that it is quite difficult to determine resistance of selected materials under natural conditions. It is necessary that an unfavorable factor (high, low temperatures, drought, etc.) manifest itself rather harshly precisely during that period of plant development, when they are most susceptible to it. According to Pfahler [19], one of these stages of ontogeny is the male gametophyte of plants, whose role should be given special attention in adaptive breeding. This means that innovative *in vitro* methods of gamete analysis can come to the rescue, allowing one to quickly select genotypes that are resistant to adverse environmental factors. They started to be used quite extensively in breeding practice [20-25, 18]. Pollen quality of any agricultural crop is the main factor in reproductive biology and determines the efficiency of both natural and artificial pollination [16]. Pollen quality is affected by many factors – high and low temperatures, drought, plant damage by diseases and pests, and even introduction of plants that lead to disruption of physiological and biochemical processes. This, in turn, provokes abnormal development of pollen grains, a decrease in its fertility and, as a result, a decrease in reproductive potential, which is reflected in the seed yield and quality of the crop [26-28]. Anthropogenic factors also have a negative impact on the pollen quality: application of herbicides, soil salinity, high levels of ozone and carbon dioxide in the air, precipitation acidity, and accumulation of heavy metals in the soil [29-34]. Taking into account increased susceptibility of male gametes to external factors and using pollen analysis, one can gain primary information about responses of genotypes to certain stress factors and identify valuable gene sources at the early stages of selection. Availability of pollen for visual assessment of its response to stress factors and ability to work out samples from many gametes makes it possible to reduce the time for assessing a large number of genotypes and to identify rare and resistant forms [18, 21]. Based on this, one of the universal methods for selecting and detecting sources of high resistance, along with natural factors, is the creation of

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artificial stress backgrounds. Their advantage is a possibility to control severity levels of an unfavorable factor for which selection is carried out, enabling one to intensify assessments and selection of the corresponding starting material and thereby speed up breeding [18]. The information presented is a fairly convincing motivation for using gametophytic assessments to evaluate the potential of the gene pool of mutant tomato forms based on responses of their pollen to various stress factors and to identify genetic sources/donors of resistance.

To comprehensively assess single- and multi-marker tomato mutant forms (125 genotypes) for resistance to abiotic stress factors (high and low temperatures and drought) in terms of a set of male gametophyte traits – pollen viability, pollen tube length, pollen resistance, pollen tube length resistance using simulated stressors in the laboratory.

Material and Methods. Mutant tomato forms of different genetic and geographical origins obtained from different countries and included in a collection of the Laboratory of Plant Genetic Resources of the Institute of Genetics, Physiology and Plant Protection were studied. In the central zone of Moldova, 125 mutant forms were studied and described: Mo 24 (*wv*), Mo 36 (*Va-2*), Mo 56 (*fu*), Mo 63 (*Me*), Mo 74 (*div*), Mo 80 (*yv*) , Mo 112 (*sp, hp*), Mo 113 (*gf*), Mo 120 (*t*), Mo 122 (*res*), Mo 136 (*alb*), Mo 137 (*aud*), Mo 147 (*Mi*), Mo 158 (*ms, sp*), Mo 162 (*u*), Mo 163 (*Ve*), Mo 304 (*bip*), Mo 305 (*d, aw, wv*), Mo 311 (*op*), Mo 316 (*gs*), Mo 324 (*Ver*), Mo 328 (*c, a, lut*), Mo 331 (*br, ch*), Mo 341 (*Wo^m*), Mo 343 (*aw, o*), Mo 350 (*vit*), Mo 372 (*ven*), Mo 377 (*oc*), Mo 379 (*ful*), Mo 385 (*br, wt, y*), Mo 392 (*coa*), Mo 409 (*nv*), Mo 414 (*hy, y*), Mo 421 (*sp, l-2, u*), Mo 432 (*Ge*), Mo 443 (*ls*), Mo 446 (*o*), Mo 451 (*sp, hp, u, ogc*), Mo 463 (*Tm^{2a}*), Mo 466 (*j*), Mo 489 (*Tm-2*), Mo 500 (*Wo^m, d, aw, o, c, r, m-2*), Mo 504 (*Wo^m, aw, bk, d, o, p, s*), Mo 516 (*sp, bl*), Mo 519 (r, c, wd), Mo 529 (*Tor*), Mo 534 (*bul*), Mo 544 (*ds/$^+$*), Mo 556 (*tp*), Mo 558 (*V-3*), Mo 561 (*Xa-2$^+$*), Mo 562 (*Xan /$^+$*), Mo 565 (*Xan-4*), Mo 570 (*cpt*), Mo 576 (*V-5/$^+$*), Mo 585 (*int, al*), Mo 588 (*aa*), Mo 589 (*apn*), Mo 593 (*dd/$^+$*), Mo 594 (*dmt*), Mo 598 (*etf*), Mo 600 (*syv*), Mo 606 (*Cu*), Mo 620 (*lur/$^+$*), Mo 628 (*ful, e, hl, a*), Mo 632 (*ag, h, t, u, l-2, e*), Mo 634 (*per, c, r, l alb*), Mo 637 (*V-2, sp, u*), Mo 638 (*V-2, c, a, u, ut, gs, gf, u, mc, y, t*), Mo 640 (*int, yg-6*), Mo 651 (*Xa-3, al*), Mo 663 (*rvt, vo d, gf , sp*), Mo 666 (*Me, wv*), Mo 670 (*ig, ltf,*), Mo 722 (*mup*), Mo 723 (*mux*), Mo 724 (*pat*), Mo 738 (*stl/$^+$*), Mo 755 (*aa, wv, d*), Mo 756 (*ru, st, sy*), Mo 759 (*bls, aut*), Mo 762 (*ful, e, ra*), Mo 779 (*ms-31, l, bu, dl, al*) , Mo 781 (*wd, marm*), Mo 786 (*inc, ag*), Mo 787 (*ms-2, a, hl*), Mo 791 (*alb, mua*), Mo 794 (*afl*), Mo 805 (*cg*), Mo 809 (*cb-2, d*), Mo 822 (*glf, spl*), Mo 833 (*imp^{dia}*), Mo 834 (*Wo^m, Ln*), Mo 835 (*Ln*), Mo 838 (*mult*), Mo 851 (*clau, di, inc, ag*), Mo 900 (*pu-2*), Mo 917 (*ta*), Mo 924 (*lg, vi, y*), Mo 952 (*bls, st*), La 1159 (*ep, obl*), La 1563 (*lp*) , La 1175 (*bls, aut*), La 2529 (*alc*), La 2644 (*sh*), La 2921 (*Del*), La 2999 (*gf*), La 3013 (*nor*), La 3179 (*B^c*), La 3535 (*at*) , La 3539 (*ug*), La 3616 (*ep*), La 3738 (*el*), La 3770 (*nor*).

Mutant tomato accessions were studied in open ground from 2014 to 2016 and in a summer greenhouse from 2017 to 2019. Plants were grown in randomly located plots in triplicate in accordance with conventional methods [35]. To evaluate them and identify valuable forms, two types of screening were used: passive and active.

Passive screening was used to study morphobiological and economically valuable traits [36]. Active screening made it possible to assess and differentiate mutant forms according to their degree of resistance to abiotic stress factors (high and low temperatures, drought) using simulated provocative stress backgrounds [18] and analyzing a set of male gametophyte traits: pollen viability, pollen tube length, resistance pollen germination and ability of pollen grains germinated under abiotic stressors to form long pollen tubes.

To determine the quality of pollen and its responses to high and low temperatures and drought, freshly collected pollen isolated from flowers of each mutant form was divided into 4 parts for the experiments according to the following design: 1. The viability of freshly collected pollen was determined *in vitro* by its germination on an artificial nutrient medium containing 15% sucrose and 0.006% boric acid [37] for 3 hours at 25°C (control) and the pollen tube length was measured; 2. Heat resistance was tested by exposing freshly collected pollen to 45°C for 8 hours; then they germinated on the above-described nutrient medium for 3 hours; 3. Cold resistance of pollen was assessed by germinating on the nutrient medium in Petri dishes in a thermostat at 6°C for 24 hours; then the germinated pollen grains were counted and pollen tube

length was measured; 4. Drought resistance was determined *in vitro* by the ability of pollen to germinate and form pollen tubes of sufficient length for fertilization on a boric acid-containing (0.006%) nutrient medium with a high concentration of sucrose (38%; simulated drought) for 5 hours [18]. In each variant, at least 500 pollen grains were assessed and analyzed by their germination and ability to form pollen tubes of a certain length. The tube length was measured in divisions of an eyepiece micrometer (d.e.m.). Pollen grains were considered germinable if the pollen tube length was at least three pollen grain diameters [37].

Based on the ratio of pollen germination and pollen tube growth in the *in vitro* experiment to the control, we judged of resistance of the male gametophyte of individual mutant forms of tomato to a stress factor P = O: K x 100 [23]. To systematize mutant forms into resistance groups, with due account for the response of pollen to each of the studied stress factors upon growing their plants in different climatic conditions, we processed the resulting extensive digital material and systematized it using traditional methods [35], which allowed us to develop resistance scales with different intervals between groups for two characteristics: resistance of pollen germination and resistance in terms of the pollen tube length (Table 1). The group interval was determined by the following ratio:

$$I = (X_{max} - X_{min})/\text{number of group} = R/k,$$

where R – Difference between the largest and smallest values of measurements separately for each characteristic.

This allowed us to determine the genotype-specific response of pollen for each mutant form to three different stress factors, taking into account the value of each of the studied characteristics for each mutant form (Figures 1, 2, 3).

Table 1.

Scale of resistance of mutant tomato forms based on the male gametophyte characteristics to the abiotic stress factors (high and low temperatures, drought)

Group No.	Resistance		Resistance degree
	Pollen germination, %	**Pollen tube length (d.e.m.)**	
	Values within groups, %		
I	0 - 11	0 - 20	Susceptible
II	12 - 23	21 - 41	Low resistant
III	24 - 35	42 - 62	Moderately resistant
IV	36 - 47	63 - 83	Resistant
V	48 - 59	84 - 104	Highly resistant
VI	≥60	≥105	Super resistant
VII	0 - 23	≥63	Low resistance of pollen and > high resistance in terms of pollen tube length
VIII	≥48	0 - 31	High resistance of pollen and < low resistance in terms of pollen tube length

Results and Discussion. The developed scales enabled us to characterize the mutant tomato forms with respect to reactions of their pollen to high and low temperatures and osmotic stress (drought) and group them by two parameters characterizing their resistance: pollen germination and the ability of pollen germinated during stress to form pollen tubes long enough for fertilization.

Analysis of the viability of freshly collected pollen (control) showed high heterogeneity within the collection of mutant tomato forms. The viability of pollen, depending on genotypic characteristics of each of the studied mutant forms, ranged widely (from 1.5% to 78.2%) when their plants were grown under the same conditions.

The scatter between mutants in terms of the ability of germinated pollen grains to form pollen tubes of a certain length is also quite wide (from 11 to 150 d.e.m.). Great differences in values of these parameters between the studied mutant forms allowed us to systematize them into groups that reflect their individual characteristics.

Of the 125 accessions studied, the lowest pollen viability (3.8% - 9.9%) was found in 24 mutant forms: Mo 158; Mo 162; Mo 163; Mo 316; Mo 324; Mo 328; Mo 343; Mo 392; Mo 409; Mo 500; Mo 519; Mo 529; Mo 562; Mo 565; Mo 600; Mo 637; Mo 638; Mo 722; Mo 755; Mo 779; Mo 851; Mo 924; La 2529; and La 3013.

In many mutants (39) (Mo 24; Mo 63; Mo 136; Mo 166; Mo 248; Mo 316; Mo 341; Mo 350; Mo 372; Mo 385; Mo 432; Mo 442; Mo 446; Mo 451; Mo 463; Mo 489; Mo 504; Mo 561; Mo 588; Mo 589; Mo 593; Mo 606; Mo 638; Mo 651; Mo 663; Mo 723; Mo 732; Mo 762; Mo 776; Mo 779; Mo 781; Mo 787; Mo 791; Mo 794; Mo 835; La 2921; La 3539; and La 3770), the pollen viability was also low (10 - 20%), but higher than in the genotypes of the previous group.

The medium level of this parameter (20.2% - 30.0%) was recorded for the following mutants: Mo 36; Mo 56; Mo 61; Mo 147; Mo 311; Mo 331; Mo 377; Mo 385; Mo 466; ; Mo 544; Mo 547; Mo 555; Mo 558; Mo 564; Mo 584; Mo 593; Mo 598; Mo 620; Mo 640; Mo 651; Mo 637; Mo 640; Mo 722; Mo 738; Mo 756; Mo 805; Mo 822; Mo 833; Mo 838; Mo 918; La 2644; La 3179; and La 3616.

Good initial viability of pollen (31.1% - 39.0%) was intrinsic to 19 mutant forms: Mo 63; Mo 74; Mo 316; Mo 341; Mo 343; Mo 395; Mo 451; Mo 509; Mo 533; Mo 632; Mo 534; Mo 556; Mo 723; Mo 732; La 1159; La 1563; La 2802; La 3535; and La 3738.

The mutants with the highest viability (40.5% - 78.2%) were Mo 122; Mo 308; Mo 316; Mo 334; Mo 378; Mo 396; Mo 406; Mo 443; Mo 460; Mo 518; Mo 585; Mo 603; Mo 649; Mo 651; Mo 724; Mo 759; Mo 900; Mo 922; Mo 952; La 3535; and La 3668.

There was no clear relationship between the initial viability of pollen and the ability of germinated pollen to form pollen tubes of sufficient length for fertilization. In some mutant forms, pollen germinated actively, but germinated pollen grains could not form long tubes, and vice versa, the percentage of pollen germination could be low, but all germinated pollen grains formed long tubes. In some mutants, both with high and low initial viability, germinated pollen grains formed tubes of various lengths, from very short to very long ones. This indicates high intrapopulation heterogeneity of pollen pools, making it possible to identify their genotypic features (in terms of characteristics of the male gametophyte) and to select valuable and desirable forms. No clear dependence on mutant marker genes carried by the mutant forms was observed either.

Classification of mutant tomato forms according to reactions of their pollen to high temperature. Screening of single-marker and multi-marker mutant forms of tomato (125 forms) using their pollen exposed to high temperature (45°C) revealed pronounced differences in high temperature effects; so the mutants were grouped using the resistance scale (Fig. 1).

Group I was the mutant forms with equally high susceptibility by both pollen parameters: Mo 316, Mo 341, Mo 443, Mo 446, Mo 555, Mo 584, Mo 585, Mo 598, Mo 606, Mo 724, Mo 756, La 3737, and La 3770. The viability of freshly collected pollen (control), depending on genotypic features of these forms, varied from 12.7% to 62.5%. The tube length variation within the group was also noted: 22 - 67 d.e.m.

For Mo446 and Mo724 pollen, high temperature treatment was lethal, despite the fact that the control viability was quite high (39.4% and 62.5%, respectively). The other accessions showed heat resistance of pollen of 2.3 - 10.1%, and resistance assessed by pollen tube length of 0 - 19.4%.

Croup II included Mo 409, Mo 442, Mo 451, Mo 466, Mo 529, Mo 547, Mo 588, Mo 589, Mo 638, Mo 722, Mo 805, Mo 838, Mo 918, La 2644, and La 3179, which also showed low heat resistance of pollen (10.3% - 19.4%), and resistance assessed by pollen tube length of 21.0 - 34.5%. After heat treatment, more germinated grains were found in the populations, which formed short tubes, and, in some cases, three pollen grain diameters long, which is considered optimal for fertilization [37]. A significant decrease in the both parameters of pollen in the

experiment compared to the control for all the studied accessions characterizes them as weakly resistant.

Medium resistance (group III) both in terms of the percentage of pollen germination (24-35%) and the ability of germinated pollen to form tubes of a certain length (22-47%) compared to the control was noted in 7 mutant forms: Mo 56, Mo 158, Mo 308, Mo 331, Mo 663, Mo 918, and La 3668. In the pollen pools of these tomato mutants, more active germination of pollen grains was detected after high temperature treatment and most of the germinated ones formed tubes of sufficient length for fertilization. According to data on the pollen response to high-temperature exposure, 29% of mutant tomato forms were considered as susceptible or very low resistant (Fig. 1).

For the selection of high-temperature resistant forms, mutants of groups IV, V, and VI are suitable according to the analyzed pollen parameters. They are considered as resistant, highly resistant or super resistant. They were distinguished by both high percentages of pollen germination after heat treatment and formation of long pollen tubes.

Pollen of mutant forms of group IV (Mo 334, Mo 385, Mo 463, Mo 561, Mo 632, Mo 651, Mo 732, Mo 851, and La 1563) actively germinated (36-47%) *in vitro* after heat treatment, forming long pollen tubes, according to which the resistance was 63.7% - 82.6%.

Mutant forms of group V (Mo 24, Mo 61, Mo 328, Mo 378, Mo 406, Mo 556, Mo 594, Mo 781, Mo 822, Mo 917, and Mo 922) were characterized by less susceptibility to high-temperature exposure. The heat resistance of pollen in the group ranged from 48% to 59%, and the resistance assessed by tube length - from 84.0 to 101.9%.

High-temperature treatment of pollen did not affect the measured parameters in ultra-resistant mutants: Mo 162, Mo 163, Mo 324, Mo 343, Mo 350, Mo 372, Mo 392, Mo 460, Mo 489, Mo 500, Mo 504, Mo 518, Mo 519, Mo 544, Mo 556, Mo 558, Mo 562, Mo 564, Mo 593, Mo 620, Mo 649, Mo 670, Mo 723, Mo 755, Mo 762, Mo 776, Mo 779, Mo 791, Mo 924, LA 2529, La 3013, and La 3539. For some of them (Mo 350, Mo 372, Mo 460, Mo 500, Mo 562, Mo 593, Mo 620, Mo 670, Mo 762, Mo 779, Mo 924, La 3013, and La 3539), the percentage of pollen germination after heat treatment was higher than in the control: from 3.5% to 78.1%. The germinated pollen of each of the mutant forms formed long, homogeneous, pollen tubes; and the tube length-based resistance in this group ranged from 80.1% to 194.5%. In some mutants, the pollen tubes were twice or more times longer than in the control. They were most numerous: 28% (Fig. 1). They are of particular interest for their further use in breeding and genetic studies as well as donors of resistance in breeding programs.

Along with mutant forms, pollen of which turned out to be very susceptible to high temperature (groups I–III), and mutants with weak responses to this stressor (groups IV–VI), mutants with high intrapopulation variability in the studied parameters of pollen were detected in groups VII and VIII (Fig. 1). High heterogeneity in the populations was noted both in terms of morphological characteristics of pollen grains and in terms of pollen germination and tube formation.

Group VII included quite a many mutants (23%) (Fig. 1), whose pollen germinated very poorly after high temperature treatment, but the germinated grains formed long tubes (Mo 36, Mo 63, Mo 74, Mo 122, Mo 147, Mo 166, Mo 248, Mo 311, Mo 341, Mo 396, Mo 432, Mo 451, Mo 509, Mo 533, Mo 565, Mo 603, Mo 637, Mo 640, Mo 723, Mo 794, Mo 833, Mo 835, Mo 900, Mo 952, La 1159, La 2644, La 2802, La 2921, La 2999, and La 3535). They were considered as susceptible in terms of pollen germination and resistant in terms of pollen tube length.

Reverse reactions of pollen to high temperature were observed in the mutant forms of group VIII (Mo 120, Mo 305, Mo 395, Mo 421, Mo 558, and Mo 576) with high percentages of pollen germination (83% - 124.3%) after heat treatment, but in this case, only small portions (3.8 - 9.4%) of germinated pollen grains formed tubes of normal length.

The pollen tube length was very variable within the analyzed pollen pools: slightly germinated pollen grains and tubes, the length of which was ¼ of the grain diameter; thin (filamentous), deformed and curved (spiral-shaped) pollen tubes with a length of 2 to 11 d.e.m., and often much shorter than 3 pollen grain diameters. Consequently, pollen of these mutant

forms, germinating massively after high-temperature treatment, was not able to form full-fledged high-quality tubes that could fertilize under natural conditions. Therefore, these mutant forms belonged to the group of susceptible in terms of pollen tube length.
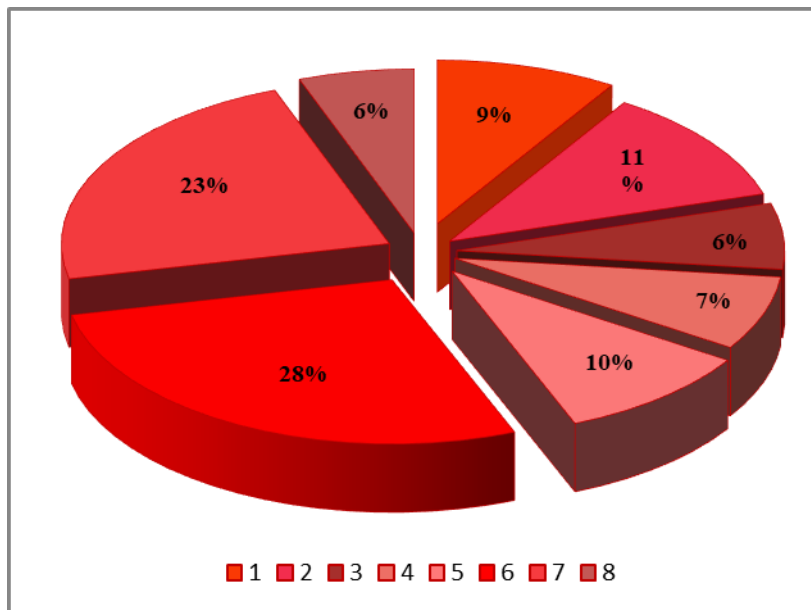

Fig 1. Grouping of tomato mutant forms by their resistance to high temperature according to the pollen parameters.
Note: **1** - *susceptible forms;* **2** - *weakly resistant by both characteristics of pollen;* **3** - *moderately resistant;* **4**. - *resistant;* **5** - *highly resistant;* 6. - *super resistant;* **7**. - *susceptible in terms of pollen germination and resistant in terms of pollen tube length;* **8**. - *resistant in terms of pollen germination and susceptible in terms of pollen tube length*

High susceptibility of these genotypes to this stressor during microsporogenesis, which leads to development of pollen grains with pronounced anomalies and, subsequently, affects their fertilizing ability, can be a possible cause of such a high heterogeneity in pollen pools in mutant forms of groups VII and VIII in terms of their ability to germinate and form tubes of sufficient length for fertilization after high-temperature treatment. High susceptibility and various viability of pollen can also be due to linkage of marker mutant genes or a pleiotropic effect of one of the alleles.

Characteristics of the male gametophyte in the mutant tomato forms at low temperature. Pollen germination of the mutant tomato forms at low positive temperature (+6°C) also showed widely variable resistance: from 0% to 130.5% for pollen germination and from 0 to 183.6% for the ability of germinated pollen grains to form tubes of a certain length. Based on the results on the individual responses of pollen of each of the studied mutant forms to low-temperature stress, we also grouped the accessions (Fig. 2) using the resistance scale (Tab. 1).

Individual analysis showed that +6°C during pollen germination was a lethal temperature for pollen of Mo 446, Mo 529, Mo 565, Mo 589, and La 2529. Strong responses to *in vitro* low temperature exposure during pollen germination and growth of pollen tubes were recorded for other mutant forms: Mo 63, Mo 316, Mo 334, Mo 350, Mo 378, Mo 395, Mo 460, Mo 504, Mo 533, Mo 556, Mo 584, Mo 588, Mo 603, and La 2999. The cold resistance of pollen in this group was 1.3 - 10.7% and the resistance assessed by pollen tube length was 3.5 - 20.8%.

At low temperature in mutant forms of group II (Mo 56, Mo 61, Mo 248, Mo 308, Mo 331, Mo 341, Mo 385, Mo 406, Mo 409, Mo 451, Mo 509, Mo 529, Mo 561, Mo 585, Mo 638, Mo 722, and Mo 762), the number of germinated pollen grains decreased by 1.5-2 times compared to the control and the resistance assessed by pollen tube length - by 1.2-2.8 times. A significant decrease in the parameters (experiment/control) characterizes these genotypes as weakly resistant. Pollen of mutant forms of group III was less susceptible to low-temperature exposure: Mo 24, Mo 328, Mo 343, Mo 396, Mo 562, Mo 723, and Mo 952 were medium resistant both by

pollen germination (24.0 - 36.0%) and by pollen tube length (42.0 - 60.6%). Quite a lot of mutant forms (34%) with strong responses of their pollen to low temperature were identified; they were referred to groups I, II and III on the resistance scale (Fig. 2).

High percentages of pollen germination at low temperature were noted both in genotypes separately and in the whole group: 36.5 - 47.9% in combination with the pollen tube length of 60.9% - 81.6% in Mo 122, Mo 136, Mo 162, Mo 163, Mo 463, Mo 564, Mo 756, and La 1159, which were assigned to group IV.

Mutant forms of groups V and VI are of the greatest interest for breeding programs as donors of cold resistance.

The following mutant forms were noticeable for high resistance to both parameters of pollen: Mo 36, Mo 74, Mo 120, Mo 158, Mo 324, Mo 489, Mo 600, Mo 606, Mo 732, Mo 787, Mo 791, Mo 822, Mo 917, Mo 851, and La 3179. The resistance assessed by pollen germination in this group was 45.6% - 57.9% and the resistance assessed by pollen tube length varied from 72.2% to 88.9%. In some forms (Mo 158, Mo 600 and Mo 851), low temperature stimulated pollen germination by 51.0% - 55.3% compared to the control and formation of long tubes by 72.0 - 82.6%, whereas in other forms (Mo 36, Mo 74, Mo 732, and Mo 822), the pollen germination and formation of long pollen tubes were similar to the control values. Here it should be noted that resistance to this stress factor did not depend on the parameters of freshly harvested pollen, since its germination at low temperature demonstrated high resistance by both pollen parameters, both in mutant forms with low and very high control values of the parameters. Consequently, the level of resistance assessed by pollen characteristics is determined by genetic features of each mutant form, which makes it possible to class them as resistant or susceptible.

Based on the pollen parameters, the following mutant forms of group VI are of particular value as sources of gene of resistance to low temperatures: Mo 311, Mo 372, Mo 392, Mo 432, Mo 443, Mo 466, Mo 500, Mo 519, Mo 534, Mo 544, Mo 547, Mo 593, Mo 598, Mo 620, Mo 632, Mo 634, Mo 637, Mo 640, Mo 649, Mo 651, Mo 670, Mo 759; Mo 779, Mo 794, Mo 805, Mo 833, M0 900, La 1563, La 3013, and La 3770. For pollen of most mutant forms of this group, low temperature had a stimulating effect, since their pollen germinated better at +6° C for 24 hours than at the optimal temperature (25°C) for 3 hours. The highest resistance by both characteristics of pollen was recorded for the following mutant forms: Mo 372 (114.6% and 141.1%); Mo 392 (102.1% and 96.9%); Mo 409 (100.1% and 97.0%); Mo 593 (119.4 and 173.5%); Mo 620 (153.4 and 136.2%); Mo 670 (127.8% and 93.8%); Mo 924 (121.6% and 148.1%); La 1563 (120.4% and 127.9%); and La 3013 (101.6% and 185.6%). They make up the largest percentage (27%) of the sample studied (Fig. 2) and are characterized as super resistant.

This may be due to physiological reorganization at the functional level, i.e. transformation of biological capabilities of organisms under influence of temperature stresses [38], which is manifested at different levels of structural organization and life activities. Low temperatures can cause a complex response, including changes in contents of proteins, including enzymes and their activities, carbohydrates and other organic compounds [39]. It is possible that high heterogeneity of the genotypes in their specific responses of pollen to different stresses is caused precisely by these changes, since these mechanisms play different roles in plant adaptation to unfavorable temperatures and may exert a weakening or, vice versa, enhancing, or protective effect.

In addition, mutant forms with high intrapopulation variability in both pollen characteristics were distinguished during germination at low temperature. They were assigned to VII and VIII groups.

Pollen of mutant forms of group VII (Mo 147, Mo 166, Mo 308, Mo 518, Mo 555, Mo 835, Mo 918, La 1563, La 2644, La 2802, La 2921, and La 3616) germinated very poorly at low temperature (1.8% - 7.8%), but germinated pollen grains formed pollen tubes long enough for fertilization (31 d.e.m. - 47 d.e.m).
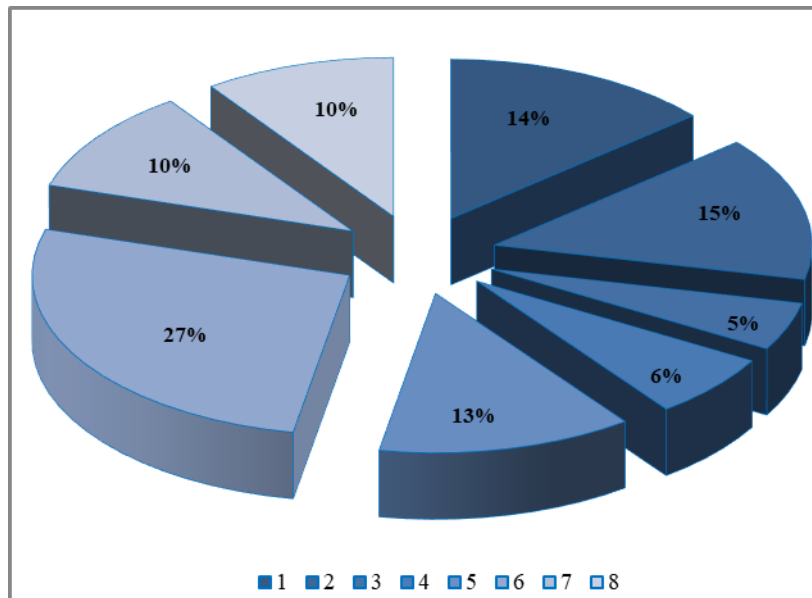
Fig 2. Grouping of tomato mutant forms by their resistance to low temperature according to the pollen parameters.

Note: **1** - *susceptible forms;* **2** - *weakly resistant by both characteristics of pollen;* **3** - *moderately resistant;* **4**. - *resistant;* **5** - *highly resistant;* 6. - *super resistant;* **7**. - *susceptible in terms of pollen germination and resistant in terms of pollen tube length;* **8**. - *resistant in terms of pollen germination and susceptible in terms of pollen tube length*

Reverse reactions were observed when pollen of Mo 113, Mo 305, Mo 377, Mo 421, Mo 442, Mo 558, Mo 576, Mo 663, Mo 781, Mo 838, La 3668, and La 3738 was studied at low temperature. Their pollen actively germinated at cold, but formed short pollen tubes. More than 50% of germinated pollen grains from the analyzed pollen pools of each of the mutants of this group had short, but very uniform in shape and length pollen tubes, equal to 1–2 pollen grain diameters. The average resistance of tubes in this group was 0.7-22.5%, with their length of 9-20 d.e.m. Such pollen under natural conditions is not able to fertilize.

The revealed high heterogeneity in the collection of mutant tomato forms by reactions of their pollen to cold stress indicates a possibility of identifying and selecting breeding-valuable forms with high resistance to this unfavorable factor for subsequent use in breeding as donors to create varieties for early seedlingless culture and unheated film greenhouses.

Reactions of pollen of the mutant tomato forms to in vitro osmotic stress. Frequent droughts in Moldova intensify genetic erosion of the tomato crop and decrease its productivity. Resistance to this stress factor is one of the most deficient and difficult ones to test [40]. Various methods are used to evaluate it and identify sources of high resistance gene: physiological, biochemical, cytological, etc. To accomplish this objective and imitate a drought, we used a nutrient medium with a high concentration of sucrose (38%) for the pollen germination and growth of pollen tubes, whose parameters are criteria of genotypes' resistance. By responses of pollen to this stress factor, the following genotypes were identified: experiencing a lethal outcome, susceptible or weakly resistant by both characteristics of pollen, resistant and super resistant in terms of germination and growth of pollen tubes, with high intrapopulation variability in pollen germination and ability of pollen grains germinated in high-sucrose medium to form pollen tubes of sufficient length for fertilization; the mutants were assigned to the corresponding groups on the resistance scale (Fig. 3). Individual analysis of responses of pollen of each mutant form to simulated drought divided a significant part of the accessions into groups I-III (40%) of susceptible genotypes and groups VII-VIII (25%) with high intrapopulation heterogeneity both in terms of morphological characteristics of pollen grains and in terms of pollen germination and formation of pollen tubes (Fig. 3).

A high concentration of sucrose had an equally strong inhibitory effect on the germination of pollen of the following mutant forms: Mo 589, Mo 722, Mo 738, Mo 851, Mo 900, Mo 918,

Mo 952, La 3535, and La 3770. They were considered as susceptible by both pollen characteristics.

Group II comprised weakly resistant forms: Mo 24, Mo 122, Mo 331, Mo 638, Mo 649, Mo 723, Mo 762, Mo 922, and La 3668. Pollen collected from flowers of these forms germinated very poorly under osmotic stress and formed tubes of insufficient length for fertilization.

The following mutant forms were classed as moderately resistant: Mo 36, Mo 341, Mo 343, Mo 377, Mo 442, Mo 606, Mo 724, Mo 756, Mo 762, Mo 791, Mo 838, and La 1563.

Mo 56, Mo 61, Mo 350, Mo 406, Mo 409, Mo 460, Mo 463, Mo 500, Mo 534, Mo 593, Mo 598, Mo 634, Mo 787, Mo 805, Mo 822, La 2644, La 3539, and La 3616 referred to groups IV, V and VI (they accounted for 34% of the sample, Fig. 3) of mutants, which were found to be resistant by both characteristics of pollen. Mo 120, Mo 372, Mo 489, Mo 558, Mo 632, and Mo 732 were characterized as highly resistant by pollen germination (48.6% - 86.0%) and tube length (60.6% - 87.3%). Zero response of pollen to this stress factor was observed in 19 mutant forms: Mo 158, Mo 248, Mo 324, Mo 500, Mo 504, Mo 519, Mo 544, Mo 562, Mo 565, Mo 620, Mo 637, Mo 651, Mo 670, Mo 779, Mo 791, Mo 924, La 1563, La 2529, and La 3013. Pollen of mutant forms of these groups demonstrated excellent germination and germinated pollen grains *in vitro* formed long tubes on high-sucrose nutrient medium. Such genotypes were significantly less numerous than genotypes with similar responses to high and low temperatures (Fig. 1 and 2)

Groups VII and VIII included mutant forms with high intrapopulation variability: in one case, in the ability of pollen grains to germinate under stress, and in the other, in the ability of germinated grains to form long tubes sufficient for fertilization. Pollen of mutants of group VII (Mo 63, Mo 136, Mo 385, Mo 392, Mo 432, Mo 509, Mo 533, Mo 640, and La 2802) germinated very poorly on high-sucrose nutrient medium.
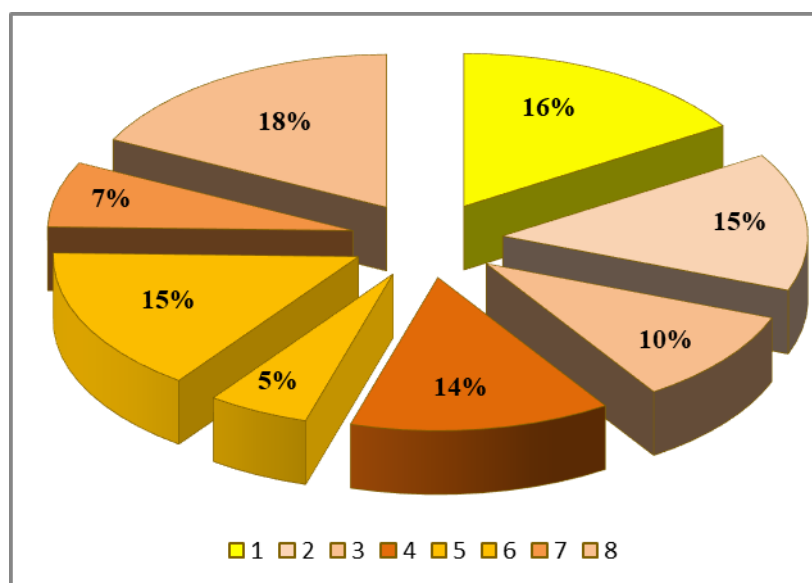

Fig 3. Grouping of tomato mutant forms by their resistance to osmotic stress according to the pollen parameters.
Note: **1** - *susceptible forms;* **2** - *weakly resistant by both characteristics of pollen;* **3** - *moderately resistant;* **4**. - *resistant;* **5** - *highly resistant;* 6. - *super resistant;* **7**. - *susceptible in terms of pollen germination and resistant in terms of pollen tube length;* **8**. - *resistant in terms of pollen germination and susceptible in terms of pollen tube length*

At the same time, a small part of germinated pollen grains formed long tubes. Pollen of mutant forms of group VIII (Mo 74, Mo 147; Mo 162, Mo 163, Mo 305, Mo 328, Mo 334, Mo 395, Mo 443, Mo 466, Mo 576, Mo 663, Mo 781, Mo 759, Mo 794, Mo 833, Mo 835, Mo 917, La 1159, La 2921, La 2999, La 3738, and La 3179) germinated actively and quickly on drought-simulating nutrient medium with a high concentration of sucrose (38%), but germinated pollen grains could not form high-quality pollen tubes of sufficient length for fertilization.

No clear relationship between resistance type and level was observed in the mutant forms for either of pollen parameters or mutant marker genes. For example, pollen of multi-marker mutant forms (Mo 500, Mo 519, Mo 632, and Mo 924) was highly resistant to all studied abiotic stressors, while pollen turned out to be quite susceptible to all stressors in others (Mo 308, Mo 451, and Mo 638).

Thus, it can be seen from the presented results that the studied mutant forms demonstrated high genetic heterogeneity in the male gametophyte traits under three simulated stresses (high and low temperatures and drought). This indicates their exceptional value for practical breeding as starting materials to create resistant tomato varieties and hybrids as well as for cytogenetic, physiological, biochemical and other studies.

Conclusions

I. Based on the results, the following groups of genotypes differing in resistance to abiotic stresses can be distinguished:

1. ***Mutant tomato accessions whose pollen is highly resistant to all studied stress factors (high and low temperatures, drought) by both pollen parameters***: Mo 324; Mo 372; Mo 463; Mo 489; Mo 500; Mo 519; Mo 534; Mo 544; Mo 593; Mo 620; Mo 632; Mo 651; Mo 670; Mo 779; Mo 791; Mo 822; Mo 924; La 1563, La 3013; and La 3539. The pollen parameters (both pollen germination and pollen tube length) reflecting their resistance demonstrated narrow ranges of intrapopulation variability under influence of different stress factors compared to the control. Pollen grains of these mutant forms germinated under stress and formed long pollen tubes; in some mutants tubes were longer than control ones. Such reactions of their pollen to stress factors make them highly or super resistant.

2. ***Mutants with equally high susceptibility of their pollen to all three stress factors***: Mo 308; Mo 316; Mo 331; Mo 341, Mo 451; Mo 584; Mo 588: Mo 606; Mo 638; and Mo 722. Strong responses of pollen to high temperature exposure was expressed in the fact that, after heat treatment, pollen of these mutant forms did not germinate on nutrient medium and few of germinated pollen grains were capable of forming pollen tubes of sufficient length. Similar results were obtained when pollen of these genotypes was germinated at low temperature or under osmotic stress.

3. ***Mutants combining high resistance to high temperature and low temperature***: Mo 162; Mo 163; Mo 392; Mo 564; Mo 600; Mo 649; and Mo 917. Exposure of pollen of these genotypes to 45ºC for 8 hours and its subsequent *in vitro* germination on nutrient medium containing 15% sucrose and 0.006% boric acid resulted in high percentages of pollen germination (44.8 - 97.3%); at the same time, germinated grains formed very long pollen tubes (48 d.e.m. - 114 d.e.m.). Germination of pollen of these accessions at low temperature (6ºC/24 hours) revealed similarly high resistance (49.0 - 87.6% for pollen germination and 50.8% - 96.0% for pollen tube formation.

As to osmotic stress, high intrapopulation variability was intrinsic to pollen, both in germination and in the ability of germinated pollen to form pollen tubes of sufficient length. In some mutant forms (Mo 162, Mo 163, Mo 600, and Mo 917), high percentage of pollen germination were noted (60.0 - 146.1%), but at the same time 67 - 92.1% of germinated pollen grains formed very short tubes (1 - 5 d.e.m.). Pollen of other mutants (Mo 392, Mo 564 and Mo 649) had small low percentages of germination (9.9% - 21.7%), but longer tubes (11-22 d.e.m.). High intrapopulation variability in two different resistance-characterizing traits is possibly explained by genetic features of these mutants, which are responsible for resistance to this stress factor.

4. ***Mutants combining resistance to high temperature and drought by both pollen characteristics:*** Mo 61; Mo 343; Mo 350; Mo 406; Mo 460; Mo 504; Mo 558; Mo 562; and La 2529. The resistance of their pollen to high temperature amounted to 45.7% - 168.6%; the resistance of pollen tubes was 80.1 - 132.1%. As to osmotic stress, the pollen germination was as good as 43.3% - 107.0%; similar results were obtained for the resistance of tubes (51.9 - 137.8%); in some accessions, values overlapped with control ones. Opposite reactions were

detected when their pollen germinated at low temperature. The resistance assessed by pollen germination in this group was 2.9% - 23.6% and the resistance assessed by pollen tube length was 8.4 - 32.3%, characterizing them as not resistant to cold.

5. ***Mutants whose pollen demonstrated high resistance to low temperature and drought***: Mo 120; Mo 158; Mo 598; and Mo 634. This is the smallest group; it comprised 5 accessions only. Pollen of these forms showed an equally high resistance when germinated both at low temperature (51.0 - 81.7%) and under osmotic stress (45.7% - 67.5%). After germination under these stressed factors, long and even pollen tubes were formed; their cold resistance was 50.4% - 85.2% and their osmotic resistance was 64.7% - 88.4%.

6. ***Mutants with high temperature resistance only***: Mo 24; Mo 328; Mo 334; Mo 378; Mo 385; Mo 518; Mo 556; Mo 561; Mo 723; Mo 755; Mo 762; Mo 776; Mo 781; and Mo 922. Analysis of the responses of pollen of these mutant forms in terms of its ability to germinate and form pollen tubes of sufficient length for fertilization under influence of different simulated stresses showed high resistance to high temperature (40.8-99.4%) by pollen germination and by pollen tube length (66.8 % - 127.3%). Pollen of these forms turned out to be susceptible to other factors (cold and drought).

7. ***Mutants with low temperature resistance only***: Mo 36; Mo 74; Mo 122; Mo 311; Mo 432; Mo 443; Mo 446; Mo 466; Mo 547; Mo 640; Mo 756; Mo 759; Mo 794; Mo 805; Mo 833; Mo 900; La 1159; and La 3179. Pollen of the mutant forms referred to this group germinated quickly and actively at low temperature and this parameter ranged from 51.4% to 141.0%, depending on genotypic features; the pollen tube length-assessed resistance was 65.0 - 127.4 %. Pollen of some mutant tomato accessions (Mo 794) germinated better at +6ºC than under optimal conditions (25ºC/3 hours).

8. ***Mutants with drought resistance only***: Mo 56; Mo 248; Mo 409; Mo 565; Mo 637; and La 2644. Pollen of these forms demonstrates excellent pollen germination associated with active growth of tubes under osmotic stress. The pollen germination-assessed resistance was 44.4% - 94.7%; the pollen tube length-assessed resistance amounted to 60.4% - 98.7%.

II. Mutant forms with high intrapopulation variability of the studied pollen characteristics were identified under different simulated stresses:

1) ***Genotypes with actively and rapidly germinating pollen grains, but with very weak growth of pollen tubes.***

2) ***Genotypes with very small percentages of pollen germination, but with ability of germinated pollen grains to form long tubes.***

The responses of the same genotype to different stress factors can be different or unambiguous. They are presented in the following subgroups:

a – High temperature exposure: Mo 36, Mo 63, Mo 74, Mo 120, Mo 122, Mo 147, Mo 122, Mo 166, Mo 248, Mo 305, Mo 311, Mo 377, Mo 395, Mo 396, Mo 421 , Mo 432, Mo 509, Mo 533, Mo 565, Mo 576, Mo 603, Mo 637, Mo 640, Mo 759, Mo 794, Mo 833, Mo 835, Mo 900, Mo 952, La 1159, La 2644, La 2802, La 2921, La 2999, and La 3535;

b – Low temperature exposure: Mo 147, Mo 166, Mo 305, Mo 308, Mo 343, Mo 377, Mo 385, Mo 396, Mo 442, Mo 466, Mo 518, Mo 555, Mo 558, Mo 576, Mo 637, Mo 663, Mo 781, Mo 835, Mo 838, Mo 918, La 2624, La 2802, La 2921, La 3616, La 3668, and La 3738;

c – Osmotic stress: Mo 63, Mo 74, Mo 136, Mo 147, Mo 162, Mo 163, Mo 166, Mo 305, Mo 328, Mo 334, Mo 385, Mo 392, Mo 395, Mo 432, Mo 443, Mo 466, Mo 509, Mo 533, Mo 576, Mo 640, Mo 663, Mo 781 Mo 759, Mo 794, Mo 833, Mo 835 Mo 917, La 1159, La 2802, La 2921, La 2999, La 3738, and La 3179.

The genotypespecific reaction of pollen of each mutant form to the action of various stress factors – high, low temperatures and drought is determined by different genetic systems, revealing differences even within a population of the same mutant form in two different traits: pollen germination and the ability of pollen grains germinated under stress factors to form pollen tubes long enough for fertilization.

From the presented results it is clear that mutant forms, as complex objects for research, demonstrated high genetic heterogeneity within the gene pool in terms of the nature of the manifestation of the characteristics of the male gametophyte (pollen). The obtained results can be used in the selection of stress-tolerant tomato hybrids.

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РЕАКЦІЯ ПИЛКУ МУТАНТНИХ ФОРМ ТОМАТУ НА АБІОТИЧНІ СТРЕСОВІ ЧИННИКИ

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У статті наведено результати скринінгу зразків мутантного генофонду томату (125 генотипів) за реакцією їх пилку на абіотичні стресові чинники (високі, низькі температури, посуху) з метою вивчення їх потенціалу та виявлення генетичних джерел/донорів стійкості. Дослідження проводили в умовах відкритого ґрунту з 2015 до 2017 рр. та в літніх теплицях з 2017 до 2019 рр. Показано високу гетерогенність генотипоспецифічної реакції пилку мутантних форм на різні стресові чинники: з летальним результатом на всі фактори (10 генотипів); висока стійкість до всіх стресових факторів (20 генотипів); комбінована стійкість: до високих і низьких температур (7 генотипів), до високих температур і посухи (9 генотипів), до холоду і посухи (4 генотипи); стійкі тільки: до високих (14 генотипів) або низьких (17 генотипів) температур, посухи (6 генотипів). Виявлено також мутантні форми (38 генотипів) з високою внутрішньопопуляційною мінливістю проростання пилку та здатністю утворювати довгі пилкові трубки за різних стресових факторів. Встановлено, що стійкість до високої температури, холоду та посухи за ознаками чоловічого гаметофіту визначається різними генетичними системами.

Ключові слова: *томат, мутантні форми, ознаки, чоловічий гаметофіт, стійкість, стресові фактори, висока температура, низька температура, посуха.*