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THE BREEDING OF HIGHLY RESISTANT TOMATO F_1 HYBRIDS TO STRESS ABIOTIC FACTORS

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Abstract. *The paper presents the results of studying the adaptive potential of F_1 tomato hybrids obtained from crossing genotypes with different combinations of mutant marker genes. The assessment of resistance to high and low temperatures is evaluated at the stage of mature male gametophyte under in vitro conditions and fruiting under in vivo conditions Genotype-specific differences in the character of manifestation of traits between F_1 hybrids, both depending on the features of the initial forms and the type of temperature stress factor, were established. A stable character of manifestation of the studied traits of male gametophyte is observed in hybrids from combinations with genotypes of cultural type and mutant forms - L8/234 × Mo835, L33/241 × Mo835, L111 × Mo 341, Fakel × Mo 341, MaKrista × Mo 341. Strong variability was characteristic of F_1 intermutant hybrids (L33/16 × Mo 835, L11069 × L28, L45/16 × Mo 341) under both stress backgrounds (45°C and +6°C) including fruiting in three different years of research (2021-2023).*

Keywords: F_1 tomato hybrids, fruit setting, male gametophyte traits, temperature stress factors, resistance.

INTRODUCTION

Creation of heterotic hybrids of tomato F_1 is one of the most priority directions of breeding programs. Heterotic power of plants, their higher productivity, early maturity, resistance to diseases in comparison with the original forms and released varieties, shows high efficiency of hybrid cultivation, both in the conditions of open and protected soil. It has been established [1] that the greatest effect of heterosis on yield and resistance to stress abiotic factors have hybrids obtained from crossing forms that differ sharply in biological and morphological traits. The crossing of such forms provides for their genetic diversity, which determines higher heterozygosity of hybrids, being the basis of heterosis. Therefore, mutant forms obtained by different methods are of particular interest in this regard [2, 3]. They are carriers of a wide range of genes, the involvement of which in the breeding process can meet the requirements for modern varieties and hybrids regarding their resistance to diseases, increased content of biologically valuable components in fruits, marketable appearance – shape, size, coloring, high transportability, etc., as well as their ability to be used in the breeding process.

Genes controlling plant growth and suppressing the growth of lateral shoots are also preferred. In addition, modern breeding technologies should be aimed at the creation and introduction into production of F_1 tomato hybrids that have not only a wide range of economically valuable traits, but also have high adaptive capabilities for cultivation under conditions with limited control of environmental parameters. The temperature factor is of special importance in this respect. Exceeding the boundary of optimum temperature leads to a sharp decrease in fruit formation due to the resulting inconsistency with the genotypic norm of the response of cultivated hybrids. This necessitates the selection of F_1 hybrids with the ability to set fruit stably in a wider range of environmental temperature regimes. The solution to such a complex problem is the involvement of a wide variety of tomato *Solanum lycopersicum* L. gene pool, including mutant forms in the breeding process, with the simultaneous use of a combination of traditional selection methods with modern techniques for working with the sporophytic and gametophytic [4] phases of plants: pollen selection and provocative backgrounds of different degrees of severity.

The aim of the research is to study and select tomato forms with higher gametophyte resistance rates *in vitro* and fruit set *in vivo*, obtained using different mutant genes in comparison with existing F_1 hybrids.

MATERIALS AND METHODS

The initial material for the research was 12 F_1 tomato hybrid combinations obtained using mutant forms of Mo 835 (*Ln*), Mo 341 (Wo^m), Mo 443 (*ls*), semi-mutant lines L 111 ($sp^{\pm}$, *nor, u, hp-1, j-2*), L11069 (sp^+, *u, nor, Tm-2a, ls, br, j-2*), L33/16 (*sp, ps-2*), L45/16 (*sp, ps*) and lines homozygous for *rin* and *nor* genes – L 28, L186, L8/234, 33|341, L201 and high-tech tomato varieties of own selection Stefani, Fakel, MaKrista. The work was carried out in several stages: identification of donors and genetic sources of the most important traits; crosses; study and evaluation of the general and specific combinative ability of parental forms; evaluation of the adaptive potential of F_1 hybrids. Plants were grown in greenhouses of the Institute of Genetics, Physiology and Plant Protection, in three different climatic years (2021-2023), on plots of 10 m^2, 35-40 accounting plants from each hybrid combination [5]. Morphological description of plants of F_1 hybrids and the degree of phenotypic expression of traits, including mutant marker traits in F_1, were carried out using well-known methods [6]. At the same time, indices of such traits as: type of plant growth, inflorescence type, number of flowers on plants and number of fruit set from them in three different years were taken into account. In laboratory conditions, F_1 hybrids were tested for tolerance to high and low temperatures according to male gametophyte traits using artificially created stress backgrounds [4]. For this purpose, freshly collected pollen from fully opened flowers of each hybrid was divided into 3 parts. One part was immediately sown on

artificial nutrient medium consisting of 15% sucrose and 0.006% boric acid and germinated under *in vitro* conditions for 3 hours at 25°C. Viability (*p, %*) was determined and pollen tube length (*l, μm*) was measured in ocular micrometer divisions, this served as control (C). The second part of pollen was used to evaluate heat resistance by treating it at 45°C for 8 h, followed by germination and determination of viability (*p, %*) and pollen tube length (*l, μm*). Cold tolerance of F_1 hybrids was determined by sowing the third part of pollen on nutrient medium and germinating it in the thermostat at +6°C for 24 hours. For each F_1 hybrid and in each research variant, at least 500 pollen grains were analyzed. Preparations with pollen were studied under a Zeiss light microscope. Resistance was judged on the basis of the ratio of parameters in the experiment (E) to the control (C). Statistical analysis allowed us to evaluate the variability (V, %) and genetic stability of the studied traits in F_1 tomato hybrids depending on the characteristics of the initial forms and growing conditions.

RESULTS AND DISCUSSION

When assessing the resistance of tomato genotypes to environmental stresses, it is necessary to take into account that the level of favorable temperature intervals for the development of reproductive organs is narrower than for vegetative organs. In this regard, the male gametophyte is of special interest. It is the most sensitive to the influence and change of environmental conditions, unlike the female gametophyte, which is covered with thick layers of somatic tissues. Therefore, pollen quality can serve as a criterion of genotypes' reaction to changes in environmental conditions, which is reflected in the reproductive ability of plants [7] and the formation of high-quality seeds [8]. Taking this into account, we evaluated the resistance of new F_1 tomato hybrids to high- and low-temperature stress factors, considering into account the nature of manifestation according to male gametophyte traits – pollen viability, pollen tube length, resistance according to pollen germination under the influence of stress temperatures and the ability of germinated grains to form pollen tubes long enough for fertilization (three pollen grain diameters).

The results obtained showed a complex character of the manifestation of the studied traits in F_1 hybrids and strongly pronounced differences between hybrids, both with respect to the reaction of their pollen to high (45°C) and low (+6°C) temperatures, and depending on the initial forms used in crosses (Tables 1 and 2). The degree of variability (V, %) of both pollen traits in individual hybrids was very high and, on the contrary, very low with respect to two different stress factors. For example, pollen from hybrids from the combinations L8/234 × Mo835, L33/241 × Mo835, L111 × Mo 341, Fakel × Mo 341, MaKrista × Mo 341 and the standard sample F_1 Ingstar responded less to high-temperature exposure. Consistently high

indicators in three different years of research (2021-2023) are confirmed by low variation coefficients for the "viability-germination" trait – 5.7-9.4% (Table 1) and the "pollen tube length" trait – 6.7-9.6% (Table 2). Similar high results for these F_1 hybrids were obtained when their pollen was germinated and at low temperature (Table 1). The average coefficient of variability for the trait "cold tolerance in pollen germination" was consistently low in three different years of the study, ranging from 3.2 to 9.6% (Table 1). The same low variability was observed for "pollen tube length", 4.4-8.2% (Table 2). This indicates the high adaptive potential of these F_1 tomato hybrids to both high and low temperatures. A high degree of variability (V, %) of the trait "pollen germination" under both stress backgrounds was observed in hybrid combinations – L33/16 × Mo 835, L11069 × L28, L45/16 × Mo 341 and Mo 443 × Fakel (Table 1).

Table 1. The influence of high and low temperatures on pollen viability-germination (p, %), of different F_1 tomato hybrids

Hybrids F_1	**Pollen germination at temperature**					
	25°C - 3 hour (control)		**45°C - 8 hours**		**6°C – 24 hours**	
	Average for three years (2021-2023),%					
	x	**v,%**	**x**	**v,%**	**x**	**v,%**
L8/234 × Mo 835	88.9	18.9	76.3	7.9	80.1	11.4
L33/16 × Mo 835	19.4	20.7	7.9	36.3	27.6	33.2
Stefani × Mo 835	42.3	7.8	20.4	10.6	64.7	9.6
L33/241 × Stefani	58.2	5.7	55.5	9.4	71.4	7.3
L186 × Mo 835	70.0	13.1	68.3	14.7	69.1	9.2
L11069 × L28	24.5	41.4	11.1	56.6	47.3	28.7
L45/16 × Mo 341	10.7	31.3	4.1	42.8	5.6	37.5
L111 × Mo 341	63.9	6.4	51.2	8.7	60.4	4.8
L 201 × Mo341	66.4	8.7	53.6	10.1	15.5	24.4
Mo 443 × Fakel	33.1	28.2	12.7	24.6	13.3	30.1
Fakel × Mo341	31.2	11.2	45.2	8.4	18.1	10.6
MaKrista × Mo 341	83.9	3.4	72.0	5.7	88.9	3.2
Standard - F_1 Ingstar	77.4	10.3	64.1	7.2	71.7	4.8

Similar response of pollen of these F_1 hybrids to temperature stressors was observed for the trait "tube length" (Table 2). The coefficient of variability for both traits (average for three years) is very high for pollen from 36.3% to 56.6% (45°C) and from 28.7 to 33.2% (+6°C), while for pollen tube length from 21.7 to 51.6% – 45°C and from 20.3 to 24.4% – + 6°C (Tables 1 and 2). It can be assumed that the high degree of variability of both pollen traits is determined by the peculiarities of parental lines 33/16, 11069, 45/16 and Mo 443, which are carriers of *ls, br, ps ps-2*

genes. The dominant character of inheritance of these genes by F_1 hybrids is a consequence of high morpho-biological heterogeneity of pollen grains in their populations. Morphological abnormalities in the structure of flowers and their individual elements, as well as the presence in the populations of these F_1 hybrids of a large number of small, puny pollen grains with disturbed integrity, and reflected in the character of the manifestation of traits both in freshly collected pollen (control) and under the influence of temperature stress factors.

In addition to those noted, F_1 hybrids with ambiguous reaction of their pollen to temperature stressors were also identified. F_1 Stefani × Mo835 pollen was susceptible to high temperature, but it germinated perfectly at low temperature, forming very long tubes at the same time. Conversely, pollen from hybrids L201 × Mo341 and Fakel × Mo341 showed high tolerance to high temperature (45°C/8 hours) and very poor germination at low temperature (6°C/24 hours) (Tabl.1 and 2).

Table 2. Effect of temperature on pollen tube length (*l, μm*) of different F_1 tomato hybrids

Hybrids F_1	**Length of pollen tubes at temperature**					
	25°C - 3 hour (control)		**45°C - 8 hours**		**6°C – 24 hours**	
	average for three years (2021-2023) in ocular-micrometer divisions (*l, μm*)					
	x	**v,%**	**x**	**v,%**	**x**	**v,%**
L8/234 × Mo 835	90.1	8.5	69.6	6.8	75.0	8.2
L33/16× Mo 835	42.0	26.4	31.2	21.7	39.6	20.3
Stefani × Mo 835	58.6	10.2	30.0	9.6	56.8	10.1
L33/241 × Stefani	71.2	6.3	64.7	8.7	77.4	4.3
L186 × Mo 835	60.3	8.1	61.5	7.4	57.4	11.2
L11069 × L28	33.4	32.7	24.2	44.9	41.3	23.7
L45/16 × Mo 341	36.2	40.3	24.8	51.6	10.0	24.4
L111 × Mo 341	67.7	7.7	47.4	9.2	52.2	5.6
L 201 × Mo341	88.7	9.3	67.3	10.7	20.0	31.2
Mo 443 × Fakel	44.1	18.4	40.2	21.2	43.6	22.3
Fakel × Mo341	40.6	14.8	48.3	6.7	25.1	19.6
MaKrista × Mo 341	64.4	4.7	61.7	8.6	71.2	7.4
Standard - F_1 Ingstar	96.2	3.9	74.1	9.7	82.4	10.2

This approach to research allowed us to identify the response of pollen of each F_1 hybrid to the action of high and low temperatures on the manifestation of two traits of male gametophyte (pollen germination and pollen tube length), which suggests its use as an express method for evaluation and selection of genotypes resistant to temperature stresses.

The study and analysis of fruit set in F_1 hybrids from different crossing combinations also showed significant differences between them. At the same time, a pronounced relationship between pollen traits and fruit formation level in hybrids from different crossing combinations should and be noted. For example, in hybrids (Mo443 × Fakel, L33/16 × Mo 835, L45/16 × Mo 341 and Fakel × Mo341) with high variability of pollen traits (Tables 1 and 2) in three different years of study (2021-2023), the number of fruit set from the number of flowers on the plant was below 50% and respectively amounted to 40.0%, 40.4%, 42.6% and 48.5%. Whereas the percentage of fruit set was higher than the standard sample (F_1 Ingstar - 84.2%) in 4 F_1 hybrids from combinations: L111 × Mo341, 85.4%; L8/234 × Mo835, 86.3%; L33/241 × Mo835, 89.5%; and L186 × Mo835, 89.9%. These F_1 tomato hybrids also showed high tolerance to high and low temperature stressors according to male gametophyte traits (Tables 1 and 2). The ability of pollen grains germinated under the influence of stress temperatures to form long pollen tubes confirms their high reproductive potential. Such a pollen tube in vivo provides fertilization, contributing to high fruit set and the formation of a large number of full-fledged high-quality seeds. Lower than the standard, but quite high fruit set (from 68.9% to 81.2%) was also observed in other studied F_1 hybrid combinations - Stefani × Mo835, L11069 × L28, L201 × Mo341, MaKrista × Mo341.

These two groups of hybrids are characterized by a wide range and original combination of economically valuable traits, including resistance to one or both high and low temperatures simultaneously. Consequently, with minimal improvement, they can be recommended for cultivation in production conditions in the Republic of Moldova

CONCLUSIONS

Genotype-specific differences between F_1 tomato hybrids have been shown, both with respect to the response of their pollen to high and low temperature stress factors and the manifestation of these traits depending on the characteristics of their original parental forms and the conditions of plant cultivation in three different climatic years (2021-2023). These results may become the basis for a deeper study and identification of the influence of various combinations of mutant genes in heterozygous offspring on the character of manifestation of traits of key importance for practical tomato breeding and, especially, in the creation of heterozygous hybrids with high indices of economically valuable traits and increased adaptability to temperature stresses.

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