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TRAITS OF TOMATO MALE GAMETOPHYTE AS A CRITERION FOR SELECTION OF BREEDING VALUABLE FORMS AT EARLY STAGES OF ONTOGENESIS

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Summary. *In vitro* assessment of pollen qualitative traits affecting reproductive and other functions of tomato plants was carried out, including under the influence of a high-temperature stress factor in multimarker lines and F_1 hybrids obtained on their basis. The F_1 hybrids and lines are characterized by morphological traits of pollen. It was revealed that the morphological heterogeneity of pollen grains in the F_1 hybrid Rozamak is associated with the dominant inheritance of the *ls* gene from the maternal form. It has been shown that the processes of pollen germination and pollen tube growth under in vitro conditions in both freshly collected and heat-treated F_1 hybrids occur synchronously and in a shorter period (1-2 hours) than in their parental lines.

Keywords: tomato, F_1 hybrids, lines, pollen grain diameter, variability, pollen germination, pollen tube growth, resistance

Introduction

Increasing plant resistance to the impact of extreme environmental factors has become one of the priority areas of both fundamental scientific research and applied breeding. Various authors have noted that environmental resistance in the plant world is one of the most deficient economically valuable traits [10, 13, 16]. It is the narrow limits of genetic diversity of environmental resistance, for example, to temperature stresses that have become the main reason why over the past 50 years there has been no significant increase in the upper level of tolerance to these factors in major agricultural crops [3]. Consequently, the creation of tomato varieties and hybrids resistant to stressful abiotic factors through breeding is one of the main conditions for the implementation of their genetically embedded potential productivity. The complexity of solving this problem is that it is very difficult to determine the resistance of the selected material in natural conditions. It is necessary that the effect of an unfavorable factor (high, low temperatures, soil salinity, plant damage by diseases and pests, plant introduction, precipitation acidity level, accumulation of heavy metals in the soil, etc.) manifests itself quite harshly precisely at that period of plant development when they are most sensitive to it. According to Pfahler (1982), one of such stages of ontogenesis is the male gametophyte of plants, the role of which in the process of adaptive selection requires special attention. This is confirmed by the high negative impact of stress temperature and anthropogenic factors on the quality of pollen, identified by various authors [4, 9, 11, 12, 14]. Considering the increased sensitivity of male gametes to external factors, pollen analysis can be used to obtain primary information on the reaction of genotypes to the action of a specific stress factor and thereby quickly and effectively differentiate them and identify valuable gene sources at the early stages of the selection process to create resistant forms of tomato [5, 17]. Studies related to environmental control of pollen development both at the stages of formation [15, 19] and functioning of mature pollen [6, 18, 21, 23] confirm its genotype-specific response to the action of certain stress factors leading to the distribution of micro gametophytes into groups. Consequently, the quality of pollen, being one of the most important factors of reproductive biology, determines the efficiency of both natural and artificial pollination processes, which in turn

depend on the characteristics of pollen formation under the influence of various stress factors of the external environment. This means that innovative technologies of gametophyte analysis, allowing for the analysis of large pollen populations in a relatively short time on artificially created stress backgrounds, in vitro can be actively used in breeding practice not only on tomatoes, but also on other crops for the identification and creation of stable genotypes [5, 7, 22].

Based on the above information, the aim of our research was to study the nature of the manifestation of male gametophyte traits that affect reproductive, reproductive and other functions of tomato plants, including under the influence of a high-temperature stress factor, in multi-marker lines and F_1 hybrids obtained on their basis.

Material and methods

The studies were conducted in 2021-2023. The object of the research was pollen (male gametophytes) of multimarker lines: L 8/234 (*sp*$^{\pm}$, *br, u, nor*); L 28 (*sp*$^{+}$, *Mi, Tm-2, rin*); L 111 (*sp*$^{\pm}$, *nor, j-2*); L 33/241 (*sp*$^{\pm}$, *rin, u, ls, ps*); L 11069 (*sp*$^{+}$, *u, nor, ls, br, j-2*) and F_1 hybrids - Ingstar, Rozамak, No. 11, obtained on their basis. The nature of the manifestation of such pollen traits as the size of pollen grains, fertility, germination-viability, pollen tube length, pollen germination resistance and pollen tube length resistance were assessed. Freshly collected pollen of F_1 hybrids and their parental forms was divided into three parts, one of which was stained with acetocarmine and morphological characteristics and fertility were determined [8]. The second was sown on an artificial nutrient medium consisting of 15% sucrose and 0.006% boric acid and germinated *in vitro* at a temperature of 25 °C for 3 hours and its viability and length of pollen tubes were determined (control). The third part of the pollen was used to assess its heat resistance by treating it with a temperature of 45 °C for 8 hours, followed by germination and determination of the ability of heat-treated pollen to germinate and form pollen tubes of a length sufficient for fertilization - three diameters of a pollen grain [1] (experiment). For each genotype in each variant of the study, at least 500 pollen grains were assessed. The reaction of F_1 lines and hybrids to the high-temperature stress factor was determined by the difference between the indices of freshly collected pollen (control) and heat-treated pollen (experiment). The size of pollen grains was calculated by measuring them using the ocular micrometer scale (μm). The dynamics of pollen grain germination (%) and the length of tubes (divisions ocular micrometer) were determined every 1, 2 and 3 hours. In genotypes with a longer pollen germination period, the counts were also carried out after 4-5 hours. All preparations were examined in a Zeiss light microscope. Statistical processing was carried out using generally accepted methods [2] and the Excel program.

Results and discussion

The conducted research allowed us to reveal quite pronounced differences in the nature of manifestation of morph biological traits (size, shape and fertility of pollen grains) of male gametophytes between F_1 hybrids and their multi-marker parental lines. The most aligned in pollen grain diameter were the populations of F_1 hybrids Ingstar (29-31 μm) and F_1 No. 11 (28-30 μm), whereas the pollen population of F_1 Rozamak was more heterogeneous with a variability of the trait index from 22 to 29 μm (Table 1). Unlike F_1 hybrids, pollen grain populations of parental lines were characterized by high heterogeneity in pollen diameter and fertility (Table 1) due to the presence of a greater number of small and medium-sized grains with deformations of varying degrees and impaired integrity. The highest heterogeneity is noted in the populations of maternal forms (A ♀) of the F_1 Rozamak and F_1 No. 11 hybrids, which are carriers of the *ls, ps* and *br* genes. These genes in different combinations affect morphological deviations in the structure of flowers or their individual elements. Probably, the high heterogeneity of pollen grains in the F_1 Rozamak hybrid and the less pronounced one in F_1 No. 11 is associated with the dominant transmission of traits controlled by these genes from the maternal lines (A ♀). These maternal lines also have a high coefficient of variability (Cv) of the “pollen grain diameter” trait, depending on both the

characteristics of their genotypes (27.3% and 12.4%) and plant growing conditions (36.6% and 24.9%), respectively (Table 1). Pollen fertility, as an indicator characterizing the presence of live pollen grains in their populations, was also the lowest. This indicates a connection between an increased proportion of morphological abnormalities in pollen grains and reduced pollen fertility. The proportion of pollen grains with morphological abnormalities of varying degrees of severity in the populations of the remaining lines is significantly lower, but higher than in the F_1 hybrids obtained with their participation.

Table 1

Morphological characteristics and fertility of tomato pollen in F_1 hybrids and their parental lines (average for 2021-2023)

Genotype	Diameter of pollen grains, µm		Proportion of pollen grains with morphological abnormalities, %	Cv,%		Fertility, %
	min	*max*		*by genotype*	*by years*	
F_1 Ingstar	29	31	4.2	5.7	7.4	91.8
A ♀	27	33	10.6	8.1	7.6	82.4
B ♂	23	28	6.4	7.2	9.7	95.4
F_1Rozamak	22	29	13.2	14.6	10.2	78.3
A ♀	20	30	32.8	27.3	36.6	53.7
B ♂	21	27	11.3	9.4	7.1	72.1
F_1 Nr. 11	28	30	6.9	4.8	8.3	86.2
A ♀	18	25	23.1	12.4	24.9	64.5
B ♂	26	31	7.3	4.2	6.8	90.6
NSR_{05}	**2.8**					

The analysis of the results on the dynamics of germination of freshly collected pollen and growth of pollen tubes (control), characterizing its fertilizing capacity, also showed significant differences between the F_1 hybrids and their parental lines. Active pollen germination *in vitro* in F_1 hybrids was observed within the first hour after sowing on an artificial nutrient medium. Most of the pollen grains (61.2%) of the F_1 Ingstar hybrid germinated within 1 hour after sowing. This indicator was slightly lower in F_1 Rozamak hybrids - 39.2%, and the lowest in F_1 No. 11 - 19.6% (Fig. 1a). The pollen grains of the Ingstar and Rozamak hybrids that germinated in such a short time simultaneously formed pollen tubes of sufficient length for fertilization - 38.8 and 39.6 divisions ocular micrometer (d.o.m.) respectively. While the germinated pollen of hybrid No. 11 formed pollen tubes significantly shorter (18.7 d.o.m.) than the above hybrids within one hour after sowing, but two hours after sowing the indicator of this trait was higher - 64.3 d.o.m. than that of Ingstar - 52.3 d.o.m. and Rozamak - 43.2 d.o.m. hybrids (Fig. 1b). These results indicate a high functional potential of male gametophytes in the studied F_1 tomato hybrids.

The activity and rate of pollen germination were lower in the parental lines. Accounting for the dynamics of germination *in vitro* showed that during the first hour after sowing, the indicator was low and varied from 0.7 to 12.6% depending on the genotype characteristics (Fig. 1a). Similar results were obtained for the ability of germinated grains to form tubes of sufficient length for fertilization, with variability from 0.4 to 18.6 d.o.m. (Fig. 1b). More active germination of potentially viable pollen grains in the lines is recorded 2 hours after sowing, and the formation of the maximum length of pollen tubes after 3 hours (Fig. 1 a, b). Longer germination of pollen *in vitro* does not lead to an improvement in the indicators, which indicates that potentially viable pollen germinates and forms long pollen tubes in the first three hours after sowing. These results can serve as a prerequisite for rapid evaluation and correct selection of parental pairs with high genetic potential for pollen quality and functionality in the process of creating heterotic hybrids F_1 and subsequent effective implementation of hybrid seed production.

Evaluation of the resistance of pollen subjected to high-temperature treatment at 45°C for 8 hours (experiment) in parental lines and their F_1 hybrids under *in vitro* conditions at different exposure times – 1, 2 and 3 hours, revealed an individual response of each genotype to the action of this stress factor. An hour after sowing, pollen grains of the F_1 Ingstar hybrid actively germinated (53.2%), but the germinated pollen grains formed tubes of insufficient length for fertilization - 22.4 d.o.m. (Fig. 2 a, b).

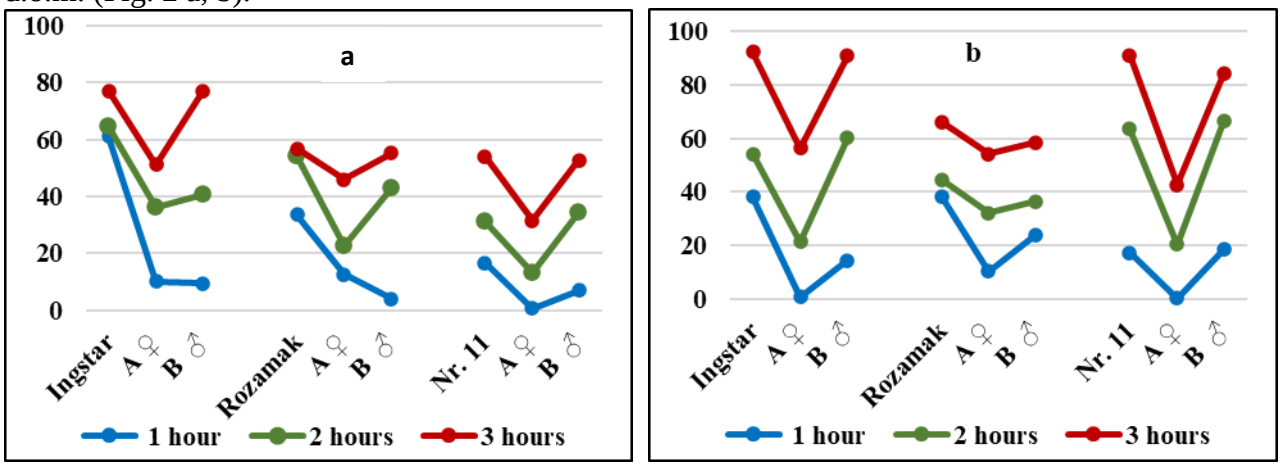


Figure 1. Dynamics of germination of freshly collected pollen (a) and growth of pollen tubes (b) in F_1 hybrids and their parental lines depending on time exposure (average for 2021-2023)

Whereas in the F_1 hybrid No. 11, fewer pollen grains germinated (18.7%), but the grains that germinated after high-temperature treatment formed long pollen tubes – 43.4 d.o.m. The F_1 Rozamak has lower indicators for both traits. As the time of pollen germination exposure increased (2 and 3 hours), the indicators of both characteristics increased proportionally (Fig. 2 a, b). The diagrams show that the F_1 Ingstar and F_1 No. 11 hybrids are characterized as highly resistant to this stress factor. The high quality of their pollen and insignificant reaction to the high-temperature factor probably provide them with high fruit set in natural conditions. For example, the F_1 No. 11 hybrid has the highest percentage of fruit set (88.4%) of the total number of flowers in all inflorescences on the plant. The F_1 Ingstar hybrid has a slightly lower indicator (81.8%) and the lowest fruit set (76.3%) is found in F_1 Rozamak. The high coincidence of male gametophyte traits with fruit set under natural conditions suggests that pollen grains of these genotypes contain a greater amount of intrinsic nutrients, which ensures rapid growth of pollen tubes and efficiency of natural pollination processes. Consequently, pollen grain germination rate and pollen tube growth rates can be used as an objective indicator for identification and selection of valuable and stable genotypes at early stages of ontogenesis in a short time.

Analysis of heat-treated pollen by the dynamics of germination and growth of pollen tubes in parental lines showed their higher sensitivity to this stress factor (Fig. 2 a, b). The pollen germination rate *in vitro* within an hour after sowing was very low and varied depending on the genotype characteristics from 6.7% to 15.4% in maternal lines, from 1.8 to 6.3% in paternal lines. Germinated pollen grains also formed short pollen tubes. Two hours after sowing pollen on an artificial nutrient medium, more active pollen germination was noted in both maternal and paternal forms. At the same time, differential differences are also manifested between the lines regarding the response of their pollen to high temperatures (Fig. 2 a, b). The highest percentage of germinated pollen grains at 3-hour exposure time was found in maternal forms (A ♀) of hybrids F_1 Ingstar and F_1 No. 11, which simultaneously formed very long tubes. Pollen of paternal lines showed higher sensitivity to heat treatment, both in pollen germination and tube length (Fig. 2 a, b). It follows that hybrid combinations, where maternal forms had higher rates for the studied traits, and hybrids are characterized by high resistance to temperature stress.

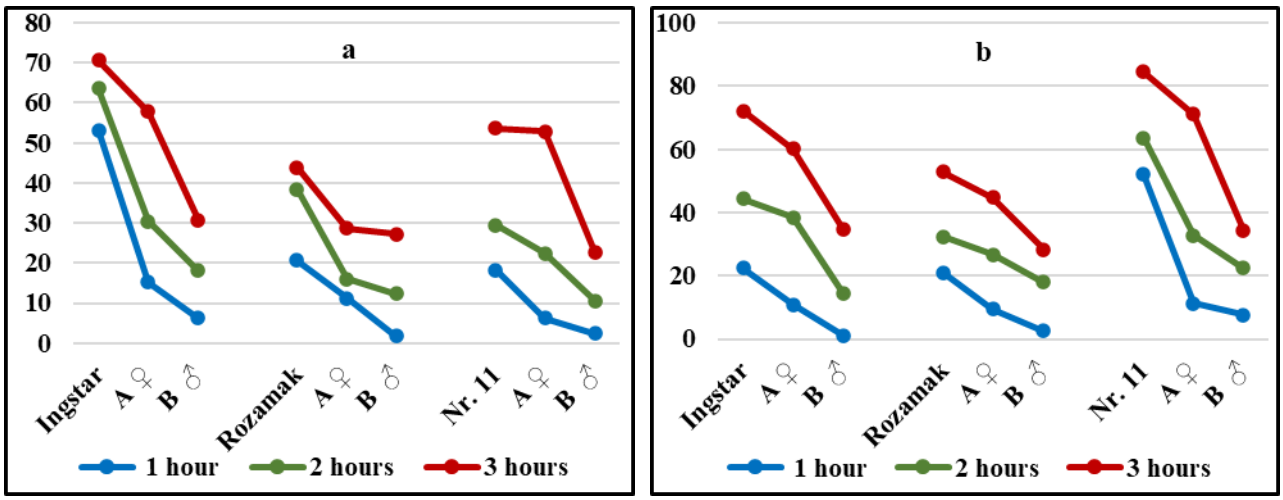


Figure 2. Dynamics of pollen germination (a) and pollen tube growth (b) after high-temperature treatment (45°C/8 hours) in F_1 hybrids and their parental lines depending on the time exposure of its germination (average for 2021-2023)

A similar conclusion can be made for individual combinations of F_1 hybrids and for the nature of the manifestation of morphological traits of male gametes. Therefore, questions concerning the nature of inheritance of the traits of the male gametophyte that we studied require a more in-depth analysis and will be reflected in subsequent scientific publications.

Conclusions

Based on the results of the research, the F_1 hybrids and their multi-marker parental lines were characterized in relation to the morphological features of their pollen grains It is shown that the pollen populations of the F_1 hybrids are more uniform in size, shape and fertility than those of the parental forms, which are highly heterogeneous due to the presence of a large number of small, medium-sized pollen grains with deformations of varying degrees, and this is especially pronounced in the lines carrying the *ls*, *ps* and *br* genes.

It was revealed that the high morphological heterogeneity of pollen grains of the F_1 Rozamak hybrid is associated with the dominant inheritance of the *ls* gene in combination with *ps* from the maternal form (A ♀), since the paternal line (B ♂) is characterized by high morphological homogeneity of pollen grains.

Taking into account the dynamics of pollen germination and pollen tube growth in both freshly collected and heat-treated pollen *in vitro*, depending on the time exposure of germination (1, 2 and 3 hours) showed that in F_1 hybrids these processes occur synchronously and in shorter periods (1-2 hours), while in parental lines they occur less synchronously and temporally longer (2-3 hours or more).

The revealed nature of the manifestation of male gametophyte traits affecting reproductive, reproductive and other functions of tomato plants, including under the influence of high temperature in multimarker lines and heterotic F_1 hybrids obtained on their basis, can serve as a prerequisite for rapid assessment and correct selection of parental pairs with high genetic potential for pollen quality and its functionality when creating heterotic hybrids and subsequent effective hybrid seed production.

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