ORGANIZATION OF THE BREEDING PROCESS AS A MEANS OF INCREASING THE EFFICIENCY OF BREEDING HETEROSIS F_1 TOMATO HYBRIDS

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Abstract

The article presents the results of a comprehensive approach to research using mutant tomato genotypes as source material and combining classical breeding methods with gametic technologies. It provides a description of F_1 tomato hybrids (10 combinations) obtained by crossing cultivated lines with mutant lines that carry marker genes (*sp^+, $sp^{\pm}$, rin, nor, alc, u, ls, br, β, gs, Wo^m, j-2*, and others) of high economic value. These are high-tech, heteritic F_1 tomato hybrids with yields ranging from 27.8 kg/m² to 36.5 kg/m² and a marketability of fruits from 89.1% to 95.6%. They are indeterminate (*sp^+*) and semi-determinate (*$sp^{\pm}$*) in growth type, with large (120...250 g) brightly colored fruits of red, pink, and orange. The high content of dry matter (5.2…6.8%), sugar (4.2…5.5%), and vitamin C (29.0…47.4 mg/%) provides high taste qualities to the fruits. A differential reaction of F_1 tomato hybrid pollen to high and low temperatures tested *in vitro* has been demonstrated. The results obtained indicate the effectiveness of heterosis breeding using complementary methods with simultaneous assessment of morpho-biological, economically valuable traits, and resistance to various temperature stress factors. This has made it possible to create F_1 tomato hybrids capable of realizing their genetically determined productivity potential in climatic conditions that are far from optimal, and to recommend them for cultivation in industrial greenhouses in the Republic of Moldova.

Keywords: tomato, breeding, methods, F_1 heterotic hybrids, economically valuable traits

Climate change and the problems arising from it are leading to the intensification of genetic erosion not only of tomatoes but also of other agricultural crops, and as a result, to a decrease in the efficiency of their cultivation (Kang M.S., 2004; Ettore Pacini & Rudi Dolferus, 2016). The negative impact of environmental factors is compounded by changes in the requirements of the vegetable consumption market, which makes it necessary to determine the relevance of tasks and new directions for breeding. Breeding is the most valuable, accessible, and cost-effective means of achieving high scientific intensity in the process of creating new tomato varieties and hybrids that can withstand the effects of adverse environmental factors and rapidly changing demands from both producers and consumers. This process should be based on an active search for sources of new germplasm with higher genetic diversity. Of particular interest in this regard are genetically and geographically distant forms, including the genetic potential of mutant tomato forms, which are a unique source of valuable germplasm for solving theoretical and practical breeding problems (Makovei, 2022). When defining the objectives and possibilities of breeding, it is necessary to identify ways to achieve them, taking into account the availability of complementary methods for rapid evaluation, identification of source material, and its effective use in creating tomato varieties and F1 heterotic hybrids capable of realizing their genetically determined productivity potential under conditions that are less than optimal. This can be facilitated by a comprehensive approach to research, combining classical breeding methods with modern gamete technologies. This makes it possible to evaluate a large amount of experimental material at the early and most vulnerable stages of ontogenesis and to select valuable genotypes that are resistant to biotic and abiotic stress factors (Makovei, 2018).

Of great interest in this regard are heterotic tomato hybrids, which could replace existing varieties created through a lengthy breeding process, thereby significantly reducing the time

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required to create new tomato varieties of better quality and higher yield.

According to existing theories (Dowker et al., 1987; Khotileva et al., 2016), heterotic hybrids are the most valuable source material for subsequent selection of genotypes with the necessary combination of traits. F1 hybrids can combine a complex of genes that determine resistance to major tomato diseases. They are also indispensable in creating forms that are resistant to abiotic stress factors such as low and high temperatures, drought, nutritional disorders and others (Khotileva et al., 2016).

Based on this, the aim of this work was to develop approaches to the use of heterosis in tomato breeding as a valuable biological phenomenon for determining conceptual research directions.

MATERIAL AND METHOD

The experimental material consisted of about 40 hybrid combinations of F_1 tomatoes obtained using specially created breeding lines with an original combination of economically valuable traits, including those controlled by mutant genes (*sp⁺, sp±, nor, rin, j-2, u, bk, gs, β*), as well as semi-mutant L11069 (*sp±, nor, br, Tm-2a, ls, j*), mutant (Mo 341 *Wo*^{m} and Mo 835 *Ln*) and lines of the cultivated type with high combinative ability – L16, L187, L202, L 454, L828, L1033, v. Stefani, and others. Plants from each F_1 hybrid combination and their parental forms were grown on 5 m² plots with 20 plants in 3 replicates at the experimental site (in a greenhouse) of the Institute of Genetics, Physiology, and Plant Protection in three different climatic years (2022-2024). The nature of the phenotypic manifestation of morpho-biological traits and the quantitative expression of economically valuable traits was determined using methods commonly accepted for tomatoes (Orzan *et al.*, 1987, Tomato-Upov). In laboratory conditions, under artificially simulated stress conditions (Makovei, 2018), F_1 hybrids were tested for resistance to high (45°C/8 hours) and low temperatures (6°C/24 hours) based on male gametophyte characteristics (pollen viability, pollen tube length – control; pollen heat resistance and tube length resistance; pollen cold resistance and pollen tube length resistance). For each F_1 combination, 20 economically valuable quantitative traits were studied. A comparative analysis of the indicators of the studied traits in the parental forms and F_1 hybrids obtained on their basis was carried out. The effect of heterosis was determined (Daskalov, 1987).

RESULTS AND DISCUSSIONS

For tomatoes grown in the ecological conditions of the Republic of Moldova, the following research areas are fundamental:

The first is the creation of F_1 hybrids with a high heterosis effect for the main breeding traits for their industrial cultivation, but at the same time, it should be noted that heterotic F_1 tomato hybrids are one of the most valuable sources of starting material for the subsequent selection of genotypes with the necessary combination of traits and the creation of new lines and varieties based on them. However, this requires the special selection of parent forms with a complex of complementary traits and high combination ability. Parent forms, differing genotypically, must have a set of desirable recessive genes. This will most likely ensure the selection of valuable genotypes from subsequent splitting hybrid populations F_2 and F_3. In our work, the progenitor of a whole range of new tomato varieties was the highly heterotic F_1 hybrid (L106152 x v. Ruslan), obtained using genetically and geographically distant forms (USA, Moldova). On its basis, a number of highly productive (from 56.4 t/ha to 73.8 t/ha) tomato varieties with varying levels and types of resistance to abiotic stress factors such as low and high temperatures and drought have been developed – *Stefani, MaKrista, MilOranj, Vivat, Matriona* and others. They are characterized by excellent taste, with fruits of various colors – red, orange, and pink. Their exceptional taste is due to their high dry matter content and balanced sugar-acid index (from 12.8 to 21). The high β-carotene content in the fruits of the *MilOranj* variety allows us to recommend it for children's dietary nutrition. These and other varieties have successfully passed testing and are homologated in the Republic of Moldova [Catalog, 2024]. For example, some of them are shown in *Figure 1 (a, b, c)*.

The use of this principle, with the simultaneous involvement in the selection process of mutant and semi-mutant tomato forms (Mo 341, Mo 443, Mo 835, L 111, L 11069) and cultivated lines (L 16, L 28, L 187, L 202, L 828, L 1033) with high combinational ability allowed us to obtain a number of homozygous lines with valuable marker traits: – 2/23; 8/234; 32/23; 33/241; 62/22; L79/22; 135/23 and 454/24. Three of them – 8/234, 33/241, and 62/22 – are being tested by the State Variety Testing Commission of the Republic of Moldova.

Figure 1 **High-performance tomato varieties *a* - Matriona, *б* - MilOranj, and *c* - MaKrista homologated in the Republic of Moldova**

They carry the *nor, rin*, and *alc* genes, which control fruit ripening and ensure high quality and uniformity in shape, size, and color, and are presented in the corresponding order in *Figure 2 (a, b, c)*. The remaining lines are undergoing competitive testing and are simultaneously used in breeding programs as donors of valuable traits for obtaining F_1 heterotic hybrids.

Figure 2 (*a, b, c*) **Homozygous tomato lines: L 8/234 with the *nor* gene; L 33/241 with the *rin* gene; L62/22 with the *alc* gene**

The next area of application for heterosis is the creation of high-yielding's F_1 tomato hybrids for industrial greenhouses. Our programs are testing more than 20 combinations of F_1 hybrids from crosses between genetically diverse lines, carriers of mutant marker genes (sp^+, $sp^\pm$, *nor, rin, alc, ls, br, j-2, u, bk, gs, β*) that control a range of economically valuable traits. The results show that 84% of F_1 hybrids exhibit a high heterosis effect in terms of productivity and fruit quality (*Tables 1 and 2*). They are characterized by high fruit set under high-temperature conditions and uniform ripening. Two F_1 hybrids named *Ingstar* and *RozaMak* (*Figure* 3 *a,b*) have been homologated and included in the Catalog of new Varieties of the Republic of Moldova (Catalog, 2024-2025). Other F_1 hybrids numbered 11, 29, 32, 39, and 54 have been evaluated in the competition test nursery and are ready to be submitted to the State Commission for further testing (*Table 1*). They produce fruits that ripen 5-7 days earlier than the early-maturing parent form, are highly productive and have good fruit quality. At the same time, it should be noted that as one of the components of crossing (usually maternal), it is necessary to involve forms with large fruits of earlier ripening, which, with an intermediate type of inheritance, balances the negative influence of the second parent. Our results show that in such crosses, it is advisable to use specially created homozygous lines with mutant marker traits (L8/234, L33/241, L187/12, and L1033) as parental forms, as they have high combinational ability. In combinations of F_1 tomato hybrids involving them, a high heterosis effect is observed in almost all cases in terms of productivity and fruit quality (shape, size, uniformity in ripening and color). They are more resistant to mechanical stress and to high and low temperature stress factors (*Table 2*). They do not have such negative characteristics as sparse placement of clusters, long internodes and tall stems, and small fruits.

Figure 3 (*a, b*) **High-tech heterotic F_1 tomato hybrids – *Ingstar* and *RozaMak, are homologated* in the Republic of Moldova and intended for cultivation in industrial greenhouses.**

Table 1

Characteristics of promising F_1 heterotic tomato hybrids according to key breeding traits (average values for 2022-2024)

F_1 hybrid number and cross combination	Vegetative period, days	Yield		Fruit mass, g	Biochemical indicators of fruits			
		total, kg/m^2	marke-table, %		dry matter, %	total sugar, %	acidity, %	ascorbic acid, mg, %
F_1 Ingstar	114	35.2	93.5	120	6.12	4.8	0.50	36.3
F_1 RozaMak	105	30.4	94.8	110	5.17	4.7	0.47	43.2
11. (L8/234 × L16)	108	30.5	95.6	140	5.21	4.4	0.51	32.7
17. (L8/234 × Mo835)	117	32.3	95.2	160	5.44	4.8	0.54	31.2
21. (L33/241×Stefani)	115	30.2	94.4	120	5.16	4.2	0.47	28.8
24. (L33/241 × L 828)	106	31.2	90.4	100	5.21	4.3	0.53	25.1
29. (L33/241 × L1033)	108	36.4	90.2	105	5.12	3.8	0.46	24.8
32. (L187 × L965)	101	33,6	91.1	90	5.74	4.9	0.48	31.2
39. (L187 × L828)	105	28.6	93.4	100	6.08	4.8	0.53	26.4
43. (L828 × L11069)	114	24.6	89.1	70	6.32	4.6	0.49	29.3
54. (L1033 × L8/234)	106	35.1	92.7	120	6.84	6.1	0.56	47.6
58. (L1033 × Mo 341)	113	27.7	93.5	110	6.23	5.5	0.58	34.4
F_1 Markiza (stand. I)	113	23.1	85.7	100	5.0	3.3	0.47	23.0
F_1 Malike (stand. II)	110	26,2	88.4	110	4.6	3.7	0.41	24.1
NSR_{05}		3.9						

Another valuable and relevant area of research is the use of heterosis in breeding for increased adaptability to biotic and abiotic stress factors. The relevance of this issue is beyond doubt, as every other year in the Republic of Moldova is accompanied by extremely high temperatures, drought, and viral epiphytotics. Breeding work in this area should begin with the evaluation of initial forms on selective backgrounds with high differentiation capacity. To this end, we use artificially created stress backgrounds (low and high temperatures) with evaluation at one of the most vulnerable stages of plant ontogenesis – mature male gametophyte (pollen). The study and analysis of pollen-based resistance traits in parental forms and their inheritance characteristics by F_1 hybrids allows for the early evaluation of a large amount of material and the selection of the most valuable and resistant traits. The results show that resistance to the studied temperature stress factors is inherited by F_1 hybrids mainly from maternal forms. Therefore, this should be taken into account when selecting pairs for crossing. Determination of the heterosis effect in F_1 hybrids for resistance to various temperature stress factors, both in terms of pollen germination and pollen tube length, revealed high indicators in almost all F_1 tomato hybrids (*Table 2*). The exception was hybrid No 43 from the L828 × L11069 cross, which involved the semi-mutant line 11069 with a complex combination of mutant genes. It is likely that the presence of the *ls, br*, and *ps-2* genes in the genome of this F_1 hybrid has a negative effect on pollen quality, which did not germinate well, and pollen grains that germinated under influence of stress factors were unable to form tubes of sufficient length for fertilization. In this combination, resistance to high and low temperatures based on the characteristics of the male gametophyte is inherited by the F_1 hybrid in an intermediate type. Knowledge of these characteristics makes it possible to correctly and more effectively approach the selection of pairs for crossing in order to obtain F_1 hybrids with high adaptive potential to the action of abiotic stress factors.

Step-by-step recording of morphological, biological, and economically valuable traits, along with simultaneous assessment of resistance to high- and low-temperature stress factors on artificially created selective backgrounds, made it possible to evaluate more than 40 combinations of F_1 tomato hybrids, determine their adaptive potential, and select the best ones. Of greatest interest are eight promising F_1 hybrids with a high heterosis effect for all studied traits. Hybrids No. 21, No. 29, and No. 54 stand out in particular, with a lower response of their pollen to stress factors and, especially, their ability to form long pollen tubes (*Table 2*). This ensures high fruit setting and, as a result, high productivity, which is confirmed by the indicators of heterosis effect on total and marketable yield (*Table 2*). They are distinguished by a developed root system, an optimal ratio of the sizes of the vegetative parts of the plant with frequent inflorescence arrangement, and high fruit setting under high temperatures with unregulated irrigation in a soil greenhouse. Of the F_1 hybrids presented in *Table 2*, five have a semi-determinate growth type with a shorter fruit ripening period (from 101 to 106 days). Plants of other F_1 hybrids are characterized by indeterminate growth type, with a longer fruit ripening period (from 108 to 117 days). All F_1 hybrids are characterized by high productivity (from 24.6 kg/m² to 36.4 kg/m²) and marketability (90.2%... 95.4%) with uniformly colored red, pink, and orange fruits. They carry marker traits that allow the hybridity of plants to be determined at early stages of growth and development, ensuring high quality and decorative appeal of the fruits and plants in general.

Table 2

Heterosis effect on the main economically valuable traits in promising F_1 tomato hybrids

F_1 hybrid number and cross combination	Traits and heterosis effect index (X, %)						
	Yield		Fruit mass	Resistance to pollen germination		Resistance along the length of pollen tubes	
	total	marketable		to > t	to < t	to > t	to < t
11. (L8/234 × L16)	167	204	154	108	99	126	94
17. (L8/234 × Mo835)	103	166	171	108	137	103	101
21. (L33/241 × Stefani)	211	278	163	101	164	132	207
24. (L33/241 × L 828)	138	182	114	103	101	167	184
29. (L33/241×L1033)	229	176	192	142	178	151	191
32. (L187 × L965)	114	132	111	98	101	104	94
39. (L187 × L828)	126	124	108	118	97	100	109
43. (L828 × L11069)	115	101	74	105	81	93	86
54. (L1033 × L8/234)	271	143	123	103	186	102	206
58. (L1033 × Mo 341)	152	131	117	104	101	107	101

Another equally important area of using for heterosis is the creation of F_1 hybrids for plastic greenhouses. Since most tomato production in the Republic of Moldova (76.3%) is concentrated in small and medium-sized farms and on private plots (Botnari & Cebotari, 2013), varieties and hybrids for cultivation in unheated plastic greenhouses are in high demand. To create them, we used an original method specially developed by us, which consists of crossing early-maturing, high-yielding breeding lines and varieties that are resistant to low positive temperatures with heterotic F_1 hybrids with high genetic resistance to various diseases (*Fr, C, Ph, ToMV*). The results of its application are quite promising. F_1 hybrids – 60/04, 60/21, 60/57, 60/84, and 60/96 were obtained, which exceeded standard samples (*F_1 Markiza* and *F_1 Malike*) in early and total yield by 1.1–3.4 kg/m² when grown in spring-summer crops in unheated film greenhouses and by 0.6–2.3 kg/m² when grown in summer. They are distinguished by uniform large fruits on all inflorescences regardless of their location on the main stem of the plant, have short internodes, frequent inflorescence arrangement, and are resistant to the most common tomato diseases. These heterotic F_1 hybrids of tomato have been tested in control and competition nurseries, where they have shown stable expression of the main economically valuable traits. They are characterized by high pollen resistance to low temperatures under natural conditions (15-20°C). This ensures high fruit setting (78.6...89.4%) when growing these plants in early the spring-summer period. They can be recommended for cultivation in unheated film-covered soil greenhouses in the ecological conditions of the Republic of Moldova.

CONCLUSIONS

It has been shown that heterosis is a valuable biological property of tomatoes, the use of which makes it possible to tap into additional reserves of the plant, increasing its yield, product quality, and resistance to temperature stress factors.

The heterosis effect is most often and clearly manifested in crossbreeding combinations with special and correct selection of parent pairs. The main principle in selecting pairs for crossbreeding is the presence of valuable recessive genes in the initial forms, the combination of which in a single F_1 hybrid genotype gives the expected heterosis effect.

New tomato varieties and F_1 heterotic hybrids have been created with an optimal ratio of morphological structures, high overall and marketable yields, combining resistance to high and low temperature stress factors and stable expression of these traits in three different years of research, which can be recommended for early cultivation in unheated plastic greenhouses and high-tech greenhouses in the Republic of Moldova.

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