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GENETIC POTENTIAL OF CULTIVATED AND MUTANT GENE POOL OF TOMATO (*Solanum lycopersicum* L.), METHODS OF RESEARCH AND USE IN BREEDING

411.04. Plant breeding and seed production

Abstract of the thesis for an scientific degree

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CONTENT

GENERAL DESCRIPTION OF WORK

Relevance of the topic. Tomato (*Solanum lycopersicum* L.) is the most consumed vegetable crop in the world [36], which has high nutritional, technological and taste qualities. Recently, breeders have made significant progress in creating highly productive varieties and heterotic F_1 hybrids, however, many issues remain unresolved, including those regarding their resistance to adverse environmental factors [42]. The urgency of the problem is enhanced by the fact that in the Republic of Moldova every 2-3 year is dry, accompanied by viral epiphytoties, which leads to intensified genetic erosion of the crop. This determines the need to develop new methods and technologies of the breeding process to create F_1 tomato varieties and hybrids that meet the modern requirements of producers and consumers. The success of developing valuable genotypes largely depends on the scientifically based choice of initial material, which can be isolated from genetic collections, and also developed artificially by involving genetically and geographically distant forms in crossings [15]. Mutant forms provided by different methods are of particular interest in this regard [14, 33]. The presence of a large number of easily identified mutant marker genes can help solve a wide and diverse range of practical breeding problems [3, 5, 21, 30]. The efficiency of using mutant tomato genes in the process of developing complex gene carriers, varieties and hybrids of tomato depends on the availability of information on the pattern of manifestation and the degree of phenotypic expression of mutant markers in the crop cultivation area [33]. Prediction of the breeding process and the efficiency of its implementation largely depend on the degree of their study and cultivation [15].

Currently, the problem of intensifying and optimizing the selection process, which involves the combined use of classical breeding methods with modern ones (molecular, gametic, biotechnologies, etc.), is very relevant. Gamete technologies are particularly interesting in this regard, which make it possible to quickly and efficiently process large populations of pollen under artificially created stress backgrounds, contributing to the intensification of the breeding process for the isolation and selection of resistant genotypes at the early and most vulnerable stages of plant ontogenesis [29, 46, 48, 50]. Studies related to the environmental control of pollen development both at the stages of its formation [40, 51, 54] and the functioning of mature pollen [19, 22, 23, 35, 41, 53, 57] indicate its genotype specific response to different stress factors leading to the distribution of microgametophytes into groups [29, 30, 35, 58]. To effectively combine the methods of classical breeding with gametic selection, a deeper knowledge of the characteristics of variability and inheritance of pollen traits by F_1 hybrids is required, depending on the level of resistance of their parental forms and the effect of environmental factors [17, 24], the share of the influence of parental forms and their interaction on the heritability of traits to high- and low-temperature stress by F_1 hybrids [28], recombination transgressive variability in F_2 and the breeding value of offspring of F_3 - F_5 generations obtained from resistant recombinants [25, 27]. Our research was devoted to solving these and a number of other important problems.

Purpose of research. Identification of the genetic potential of cultivated and mutant forms of tomato at the gametophytic and sporophytic level by analyzing the variability and heritability of resistance to abiotic stress factors and other economically valuable traits for the development of new initial material and highly productive varieties and hybrids.

Research objectives:

- Develop and optimize artificial stress backgrounds (high and low temperatures, drought) for screening the resistance of mutant and other tomato samples based on the traits of the male gametophyte under *in vitro* conditions.
- Study the genetic potential of cultivated and mutant tomato gene pools as a source of new germplasm for resistance to stress abiotic factors with a simultaneous assessment of the pattern of manifestation and the degree of phenotypic expression of biological, economically valuable and mutant marker traits, to differentiate and classify them.
- Conduct multicomponent crosses, including using genotypes that carry mutant genes to create hybrid populations, study them and develop effective methods to be used in breeding.
- Study the regularity of variability and inheritance of biological, cytochemical and other pollen traits by F_1 hybrids, which determine resistance to abiotic stress factors, with subsequent optimization of methods for selecting parental forms to obtain genotypes resistant to high and low temperatures.
- Estimate the share of influence of parental components and their interaction on the heritability of the traits of heat and cold resistance of pollen by F_1 hybrids.
- Develop a method for selecting transgressive recombinants from splitting F_2 populations based on the traits of pollen heat resistance and pollen tube resistance, and determine the breeding value of the offspring of resistant recombinants in the F_3-F_5 generations.
- Develop methods of combined selection in combination of traditional classical selection with gamete technologies.
- Based on the selection and assessment methods developed, produce:

a) new generation initial material with a high potential for resistance to one or simultaneously 2 - 3 stress abiotic factors in combination with a complex of other economically valuable traits for different areas of breeding;

b) tomato initial material with mutant marker traits for utilization in heterotic breeding of both open and protected soil;

- Develop new tomato varieties and hybrids with different combinations of economically valuable traits, including mutant markers and resistance to abiotic stress factors.

Research hypothesis. The presence of genotypes with different resistance to abiotic stress factors in combination with other economically valuable traits in the cultivated and tomato mutant gene pools, their assessment and study will make it possible to develop a methodology for selecting parental forms for hybrid combinations, to reveal their genetic potential of variability and inheritance of traits in order to produce valuable initial material, new varieties and heterotic hybrids of tomato.

Research methods. The research is based on methods of cultivating tomato pollen in vitro [8] to assess resistance to abiotic stress factors (high and low temperatures, drought) [29], the pattern of variability and inheritance of these traits, recombination and transgressive variability [11, 12, 14]. The pattern of the manifestation and the degree of phenotypic expression of mutant genes were studied according to the rules of tomato gene nomenclature [14, 59]. Classical crosses were carried out as simple, reciprocal, complex stepwise crosses. Cytological preparations were made to study the morphological and cytochemical characteristics of the nuclei of generative and vegetative cells of tomato pollen. [18]. The samples were classified according to the stability scale we developed [29]. The influence of combined selection using traditional methods and gamete technologies on the intensification of the tomato breeding process was studied [29]. For statistical processing of data and their graphical presentation, dispersion, cluster, correlation and other computer software packages were used Statgraf, Statistica 7, Excell.

Basic scientific provisions submitted for defense:

- Development and optimization of artificial stress backgrounds (high and low temperatures, drought) for diagnosing the resistance of tomato samples based on the traits of the male gametophyte under *in vitro* conditions.
- Genetic potential of cultivated and mutant tomato gene pools as a source of new germplasm for a complex of economically valuable traits, including resistance to abiotic stress factors (high and low temperatures, drought).
- Regularities of inheritance of resistance to abiotic stress factors according to the traits of the male gametophyte (pollen viability, resistance to high and low temperatures by pollen germination and tube length) in F1 and analysis of splitting offspring of F2 and F3 generations, etc.
- Theoretical justification for the selection of parental forms in multicomponent crosses to obtain tomato hybrid offspring resistant to abiotic stress (high, low temperatures) based on the traits of the male gametophyte: resistance to pollen, the length of pollen tubes, morphological and cytochemical traits of the nuclei of V and G cells of tomato pollen.
- Integrated use of pollen and traditional breeding methods and its impact on the intensification of the breeding process.
- Initial material for heterotic breeding of tomato.
- New varieties, F_1 hybrids and tomato lines, including multi-marker ones.

A new scientific direction of research put forward for defense: Development and employment of gametophytic technologies at all stages of the breeding process to increase the efficiency of identifying and selecting genotypes with high levels of resistance to abiotic stress (high and low temperatures and drought) in combination with pronounced phenotypic characteristics of the sporophyte.

CONTENT OF THE THESIS

INTRODUCTION. The rationale for the direction of research is presented, a brief description of the work is given, including the purpose, objectives, hypothesis and research methodology. The scientific and practical novelty of the results obtained, their practical significance and the provisions put forward for defense are shown.

1. ANALYTICAL REVIEW OF LITERATURE ON THE TOPIC OF THE DISSERTATION

It is shown that the problem of tomato breeding for environmental plasticity remains one of the key areas of fundamental and applied research. The work demonstrates growing interest in the development of varieties and hybrids that combine in the genotype the traits of early ripening, yield, fruit quality and resistance to abiotic and biotic stresses. There is a steadily growing interest in the search, identification, selection and development of new genetic sources, their replenishment of gene pools, which will allow solving many issues of practical tomato breeding. There has been a high interest in the use of marker-based, gamete and other modern methods in combination with classical ones, which help bring tomato breeding to a qualitatively new level. All these support the relevance and scientific validity of our chosen direction of research.

2. MATERIALS AND METHODS

The location and conditions of growing experimental material were taken into account when conducting research. The methods used in the process of conducting experiments at different stages of plant ontogenesis are presented and described in detail. Artificial stress backgrounds (high and low temperatures, drought) have been developed and optimized for screening and studying the genetic potential of cultivated and mutant tomato (*Solanum lycopersicum* L.) gene pools based on pollen traits [29]. A method has been proposed for determining the adaptive potential of tomato genotypes to high temperatures [26]. A resistance scale has been developed taking into account the criterion of two traits of the male gametophyte, which allows us to differentiate and systematize the results of screenings of extensive collections according to the type and degree of expression of the traits that determine resistance (Table 1).

A stress resistance coefficient has been developed [47], which combines two parameters in one index and gives a complete picture of the resistance of the genotype to a specific stress factor.

$$\boldsymbol{Kstr = \frac{Pex \times Lex}{Pc \times Lc} \times 100\%}$$

where: $\mathbf{K_{str}}$ is the stress resistance coefficient, expressed in %; **Pex** is the number of germinated pollen grains in the experiment, pcs.; **Lex** is the length of pollen tubes in the experiment (d.e.m.); **Pc** is the amount of sprouted pollen in the control, pcs.; **Lc** is the length of pollen tubes in control (d.e.m.).

A scheme has been developed for organizing work with the gene pool of mutant forms of tomato, which covers all stages and phases of the life cycle of each mutant genotype (Fig. 1).

Table 1. Resistance scale taking into account the criterion of two traits of the male gametophyte: *resistance for pollen germination and pollen tube length*

Group No	Resistance		Genotype resistance characterization
	Pollen germination, %	Length of pollen tubes (d.e.m.)	
	Values within groups, %		
I	0 - 11	0 - 20	Non-resistant
II	12 - 23	21 - 41	Weakly resistant
III	24 - 35	42 - 62	Relatively resistant
IV	36 - 47	63 - 83	Resistant
V	48 - 59	84 - 104	Highly resistant
VI	60 and >	105 and >	Ultra-resistant
VII	0 - 23	63 and >	Low pollen resistance and > high resistance along the length of the pollen tubes
VIII	48 and >	0 - 31	High pollen resistance and < low resistance along the length of the pollen tubes

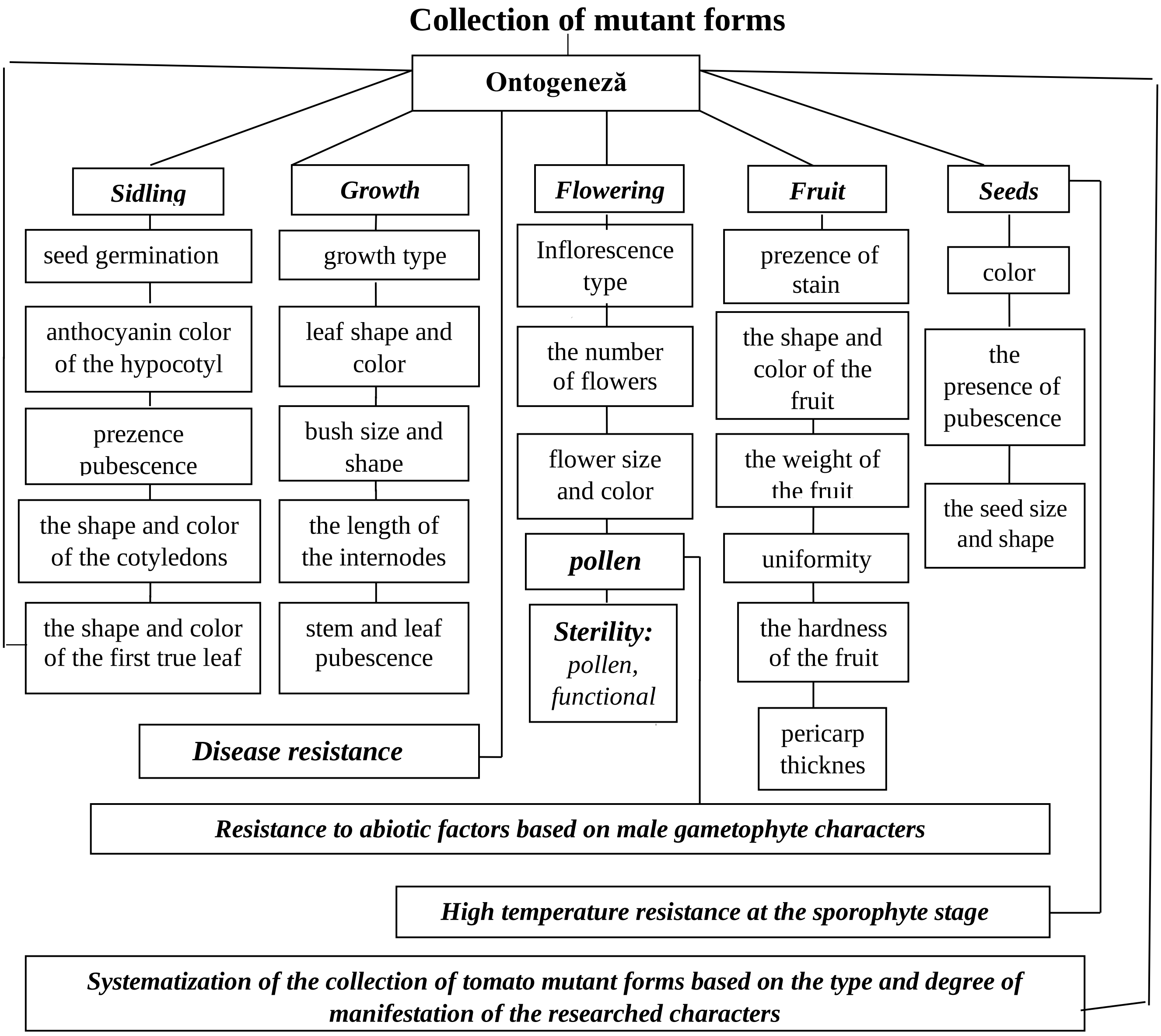


Fig. 1. Scheme of organizing work with a collection of tomato mutant forms

3. SELECTION OF TOMATO GENOTYPES RESISTANT TO STRESS TEMPERATURE FACTORS BY MICROGAMETOPHYTE

The results of screening collection material for resistance to high and low temperatures at one of the most vulnerable stages of ontogenesis, the mature male gametophyte (pollen), are shown. Screening of collection and breeding tomato samples (85 genotypes) for high temperature stress was carried out using the regime of exposing their pollen to a temperature of 45˚C for 6 hours. A high individual response of pollen of each genotype to the studied factor was revealed.

The stress resistance coefficient we developed allowed us to systematize and distribute them into groups corresponding to the type and level of resistance (Table 2). The genotypes of groups 4 and 5, characterized as resistant, highly resistant and super-resistant, respectively, are of the greatest interest. They are promising for employment in breeding as resistance donors.

Table 2. Traits of cultivated tomato gene pool samples according to the degree of their resistance to high temperature stress

Tolerance group	Treatment	Pollen viability, %	Pollen resistance, %	Pollen tube length, (d.e.m.)	Resistance along the pollen tube length,	Coefficient Stress resistance
1. Non-resistant	Control	30.2 ± 2.4		79.0 ± 1.1		
	Experiment	9.3 ± 0.7	30.8	32.5 ± 0.4	40.5	12.7
2. Relatively resistant	Control	37.8 ± 1.4		59.0 ± 1.3		
	Experiment	22.5 ± 1.2	53.3	50.0 ± 0.9	83.9	44.7
3. Resistant	Control	31.0 ± 2.0		52.0 ± 0.5		
	Experiment	25.4 ± 2.2	81.9	47.0 ± 0.7	84.6	72.0
4. Highly resistant	Control	27.3 ± 1.7		32.5 ± 0.3		
	Experiment	25.3 ± 2.0	87.6	31.5 ± 0.8	97.0	84.9
5. Ultra-resistant	Control	25.2 ± 1.4		30.0 ± 0.6		
	Experiment	25.3 ± 1.7	100.0	32.5 ± 0.9	108.3	116.6

96 tomato samples were assessed for resistance to low temperature stress by germinating their pollen at a low positive temperature of +6˚C for 24 hours on an artificial nutrient medium containing 15% sucrose and 0.006% boric acid under in vitro conditions. The percentage of pollen germination in the studied genotypes varied greatly - from 1.4% to 76.2%. This scatter shows that different samples react ambiguously to a given stress factor during pollen germination and pollen tube growth. Based on the reaction of the studied samples to cold stress, most (59 genotypes) were allocated to the first three groups: unstable, low- and moderately resistant samples. The limits of trait values within groups are very high (Table 3). At the same time, genotypes with high intrapopulation homogeneity in pollen germination and pollen tube growth were identified, which characterizes them as highly and super-resistant (groups 4 and 5).

The influence of pollen storage time at low temperatures on its qua**lity.** We studied the ability of tomato pollen (16 genotypes) to retain the optimal percentage of viable and functional pollen during long-term (60 days) storage at low temperatures (+6˚C). It was found that individual genotypes retained high pollen viability (10.0-

12.0%), which, upon germination after long-term storage, formed long tubes (28-36 d.e.m.) [49]. This indicates the effectiveness of using this method for selecting stable breeding valuable forms of tomato based on microgametophyte characteristics. It can also be widely used in industrial seed production of heterotic tomato hybrids if it is necessary to store pollen for repeated use in pollinations.

Table 3. Resistance groups according to the response of tomato genotype pollen to low temperature (+6°C/24 hours)

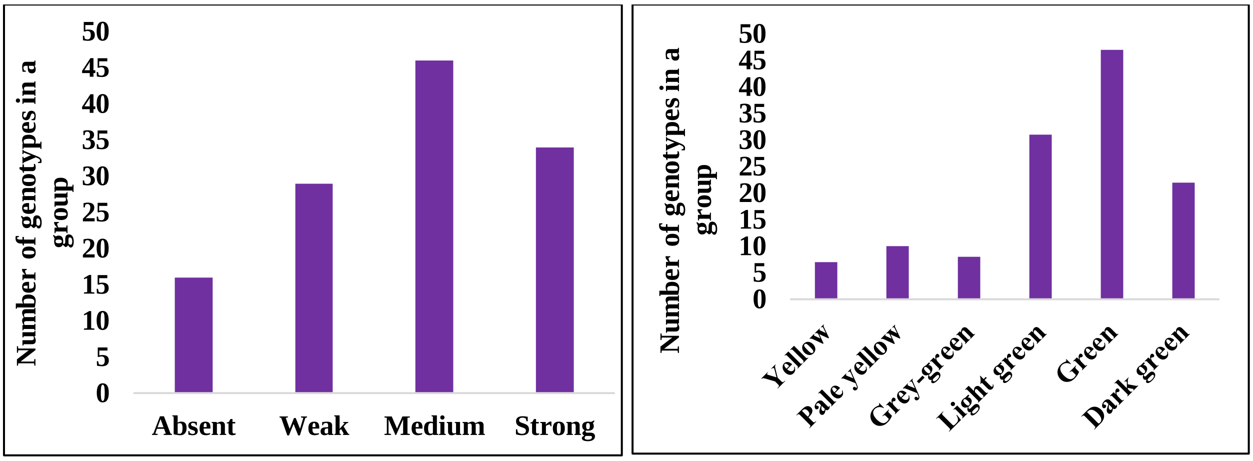
Resistance group	No genotypes per group	Range of resistance factor values per group
1. Non-resistant	28	0 - 21
2. Relatively resistant	31	28- 50
3. Resistant	15	51 - 73
4. Highly resistant	12	78 - 100
5. Ultra-resistant	10	101 - 132

4. GENETIC POTENTIAL OF MUTANT FORMS OF TOMATO AS A SOURCE OF NEW GERM PLASMA BY BREEDING VALUABLE TRAITS

4.1. Particular features of manifestation and degree of phenotypic expression marker genes at the early ontogenetic stages.

The results of a comprehensive study of the features of the manifestation of marker traits controlled by mutant genes at different stages of ontogenesis, *seedlings, growth, flowering, fruit, seeds,* are presented. *Marker genes that control anthocyanin synthesis,* identified on the day of emergence by the color of the hypocotyl, are described. Their early identification makes it possible to select the desired genotypes, to control the hybridity of seedling material and, thereby, significantly reduce the volume and duration of experiments. A group of mutant forms (16 genotypes) was identified, the hypocotyl of which lacks anthocyanin coloring; it included: Mo 588 (*aa*); Mo 305 (*aw)*; Mo 343(*aw*); 581(*ag*); Mo 585(*al*); Mo 651(*al)*; Mo 787 (*a, hl*); Mo 952 (*bls*) and multi-marker mutant lines: Mo 500 (*wo, d, aw, c, m-2*); Mo 504 (*aw, bk, d, o, p, s, wo*); Mo 632 (*ag, h, t, u, pl, e*); Mo 638 (*V-2, c, a, u, ut, gs, gf, u, ms*); Mo 755 (*aa, wv, d*); Mo 779 (*ms-31, l, bu, dl, al*); Mo 851 (*clau, di, inc, ag);* Mo 924 (*lg, vi, y).* A strong anthocyanin pigment was observed on 32 mutant forms (from intense purple to dark burgundy, turning into black), the rest were characterized by varying degrees of manifestation of the trait (Fig. 2, *a*). The high heterogeneity of mutant forms in terms of the specific manifestation of the trait indicates the advisability of their active involvement in breeding and genetic research [30].

The study and analysis of the nature of the ***manifestation of marker genes of cotyledons and the first true leaves*** within the gene pool also revealed their great diversity (Fig. 2, *b*). Differences in the degree of disturbance in shape, color of cotyledons and first true leaves (bright yellow, yellow, white-yellow, yellow-green, gray-white, light green, etc.) are controlled by a large number of mutant genes: – *aut,* *apn, alb; afl, gil, Cu, cg, c, dt, fu, inf, inta, lur/+; lut; ltf; lg-2, Me, marm, marm2, marm3, nv, oc, Op, pu2, pl, res, ru, sf, sy, syv, Tor, ver, vo, V-5/+, wv, Xan/+; Xan-4.*



a ***b***

Fig. 2. Distribution of tomato mutant forms into groups according to:
(a) – manifestation of anthocyanin on the hypocotyl; b) – the cotyledon leaf color

Variations *in shape*, ***color, type of leaf blade, degree of dissection, the presence of lobes and lobules between the main leaves, as well as corrugation and degree of pubescence*** in an adult plant are controlled by the following genes: *fu; me; div; dt; res; aut; d; Op; m-2; coa; Ver; inc; c; a; lut; ch; marm; wom; Ven; wt; nv; l-2; wd; Xan/+; Xa-3; bls; ful; mua; alpha; spl; ag; ta; inta; apn; lur; pl; per; ug-6; clau; oc; alb; ig; vo; etf; lg; Tor; bul; V-3; hl; syv; Cu; e; ra*. Following the pattern of the manifestation and the degree of phenotypic expression of these traits, for the effective use of mutant forms, carriers of these genes in breeding, they are divided into appropriate groups (Fig. 3).

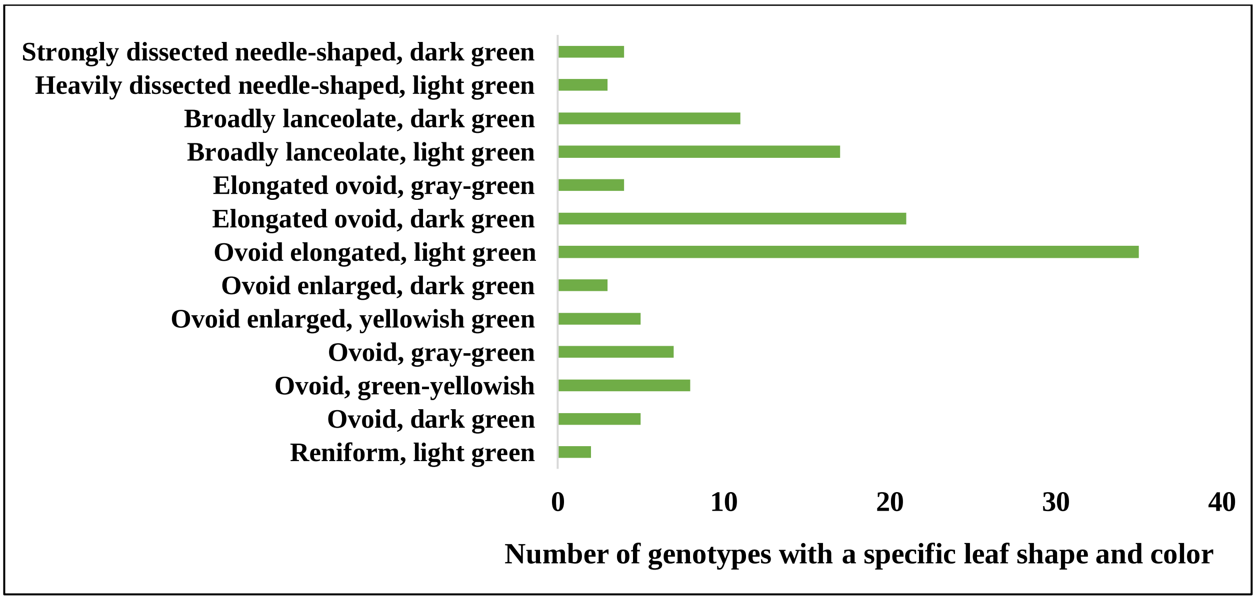


Fig. 3. Distribution of collectible mutant forms of tomato according to shape and leaf color

Traits of the reproductive system and mutant genes that control the pattern of their manifestation. The differences between the mutant forms in the size of the flower, the number of its parts, the ratio of the calyx lobes and the number of corolla lobes, their length (of the flower petals and calyx), the presence of pubescence and the color of the flowers, which are the main distinguishing features of the studied mutants, are quite pronounced. The collection includes mutant forms with highly branched *(s, mult, mup, mux, mua)*, reduced *(hg, di*), single-flowered (*uf*) and fasciated inflorescences. Mutant forms with different types of sterility have been identified and described – Mo544 (*ds*), Mo(*ex*), Mo 432 (*Ge*), Mo638 (*ms*), Mo787 (*ms-2*), Mo779 (*ms-31),* Mo732 (*psu*), Mo504 (*s*), Mo732 (*ste*), Mo738 (*ste*), Mo756 (*st*), Mo822 (*spl*). They exhibit significant variability in the number of perianth and androecium elements. The calyx lobes may be shorter in length than the corolla lobes, equal to, or significantly longer than them. These genes are of particular interest for their targeted and active involvement in the process of creating sterile forms of tomato for heterotic selection. According to the degree of phenotypic expression of the traits of the reproductive system, samples of the mutant gene pool (125 forms) were differentiated and systematized into groups (Table 4).

Table 4. Diversity of tomato mutant forms according to the manifestation of the reproductive system traits

Trait manifestation degree	**Trait value range**	**No genotypes per group**
The level of the first inflorescence fixation		
The leaf above which the first inflorescence is fixed	4 - 5	15
	6 - 8	33
	9-12	59
	13 and above	23
Inflorescence type		
Solitary flowers	1 - 3	6
Simple inflorescence	4 - 10 flowers per inflorescence	59
Simple inflorescence with strong pubescence	1 - 7 flowers per inflorescence	11
Intermediate (1-2 axis)	10 – 18 flowers per inflorescence	32
Compound (3-4 or more axes)	19 and up (up to 60 flowers)	15
Flower size and color		
Few in components – five elements	Small, light yellow, weak pubescence	3
	Small, light yellow, with pubescence	6
	Large, light yellow, without pubescence	13
	Large, light yellow, with pubescence	11
	Small, yellow, with pubescence	15
	Large, yellow, with pubescence	41
Many components – more than five elements	Small, yellow, without pubescence	12
	Large, yellow, with pubescence	24

4.2. Genotypic response of pollen of tomato mutant forms to the action of abiotic stress factors.

The results of the first assessment of the resistance of mutant forms of tomato (125 genotypes) under artificially created stress backgrounds (high, low temperatures, drought) according to a set of traits of the male gametophyte are presented: pollen viability, pollen tube length, pollen germination resistance and pollen tube length resistance. Analysis of the genotype-specific response of pollen of mutant tomato forms to the action of various stress factors made it possible to differentiate and systematize them into the group corresponding resistance scale (Fig. 4). Under different stress backgrounds, the same mutant forms responded ambiguously to the action of the stress factor. For example, with a lethal reaction to the action of one stress factor, while high resistance is recorded against the background of a different one, and on the third one, high intrapopulation variability is revealed in the pattern of pollen germination and the ability of pollen grains germinated against the background of stress to form tubes of sufficient length for fertilization.

The results of assessing samples of the mutant tomato gene pool based on the pattern and degree of manifestation of male gametophyte traits, characterizing their resistance to various stress abiotic factors, made it possible to identify mutant forms of several genetically determined types:

– Mutant forms of tomato, **characterized by high pollen resistance to all studied stress factors (high, low temperatures, drought).**

– Mutant forms, **with an equally high response of their pollen (sensitivity) to the action of the above stress factors.**

– Mutants that **combine resistance to high and low temperatures.**

– Mutants that **combine resistance to high temperature and drought.**

– Mutants that **combine high resistance to low temperature and drought.**

– Mutant forms whose pollen is **resistant only to high temperatures.**

– Mutant forms with highly resistant pollen **only to low temperatures.**

– Mutant forms, the pollen of which is highly resistant **only to drought.**

– Mutant forms of tomato **with high intrapopulation variability of the studied pollen traits under different artificially created stress backgrounds:**

***a*)** genotypes *in which, against the background of stress factors, a small number of pollen grains germinate, but the germinates form very long, uniform pollen tubes;*

b) genotypes *with actively and rapidly germinating pollen grains, but pollen grains germinating against the background of stress factors, cannot form pollen tubes of sufficient length for fertilization (three diameters of pollen grains)* [8], *(tubes are very short with deformations of varying degrees of severity).*

This allows us to conclude that the resistance to three different stress abiotic factors (high, low temperatures, drought) *is determined by different genetic systems, revealing differences even within one mutant sample in two different pollen traits: germination and the ability of germinated pollen grains to form tubes of a length sufficient for fertilization.*

The research has not found any dependence of the type and level of resistance for pollen traits on marker genes carried by mutant forms. For example, some multi-marker mutants – Mo 500 (*Wo*^{m}, *aw, d, o, r, m-2*); Mo 519 (*r, c, wd*); Mo 632 (*g, h, t, l-2, u, pl, lg*); Mo 924 (*uf, vi, y*) are characterized by high pollen resistance for both traits to all factors, while others – Mo 308 (*a, c, d, l*); Mo 451 (*sp, hp, u, og*), Mo 638 (*a, v-2, c, u, y, t, gs, gf, r, mc*); Mo 663 (*rvt, vo, d, gf, ssp*) demonstrate very high sensitivity both for pollen germination and its ability to form long tubes under all artificially created stress backgrounds. Analysis of the influence of environmental conditions on the degree of variability of pollen traits when growing mutant tomato plants in different years (2011-2014) showed an ambiguous reaction, however, the trait indicators remained within the normal reaction range characteristic of a specific genotype. This indicates the feasibility and effectiveness of using artificial stress backgrounds for the assessment, identification and selection of resistant genotypes based on the characteristics of the male gametophyte at the initial stages of plant development and the breeding process.

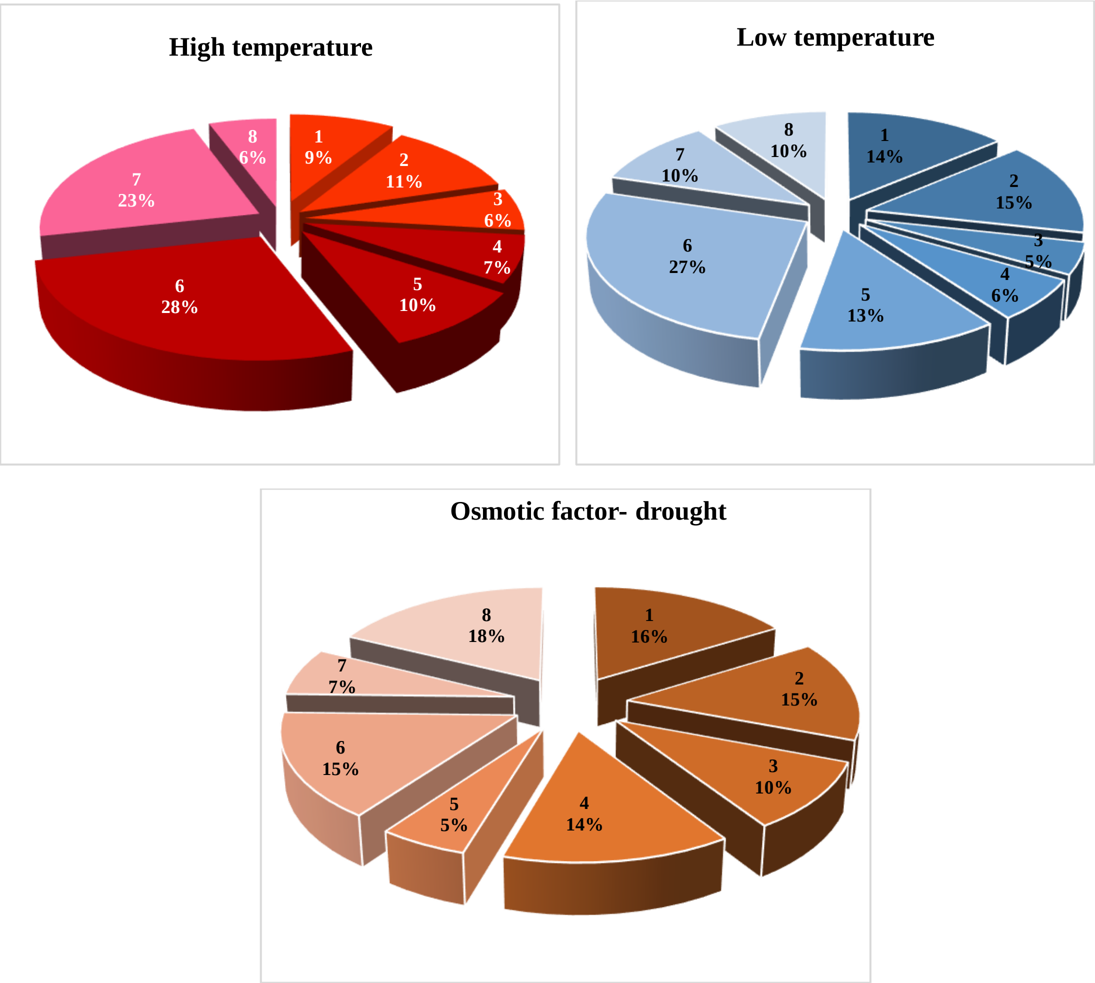


Fig. 4. Distribution of mutant forms of tomato into groups according to the degree of resistance of their pollen to high, low temperatures and drought

Note:1 – *unstable forms;* **2** – *pollen weakly resistant for both characteristics:* **3** – *moderately resistant;* **4** – *stable;* **5** – *highly stable;* **6** – *super stable;* **7** – *forms unstable in terms of pollen germination and stable in terms of the length of pollen tubes;* **8** – *resistant pollen germination and unstable pollen tube length*

4.3. Potential of mutant forms for economically valuable traits

The variety in bush shape is mainly related to the size of the plant *(d, dd, dmp, dmd, ssp, sp, sp*$^{+}$*, sp*$^{\pm}$*),* the number of lateral shoots (*ls*), the degree of their branching *(atn, bu, br, cg, bip, com, sd, bls, wd, etc.).* Taking into account the nature of the manifestation and the degree of phenotypic expression of the traits controlled by these genes and the duration of the growing season, we systematized the mutant forms into groups:

indeterminate: *early ripening* (98-108 days), *mid-ripening* (110-118 days), *late ripening* (121-134 days). day); ***determinate:*** *ultra-early ripening* (89-99 days), *early ripening* (104-109 days), *mid-ripening* (110-118 days), *late ripening* (121-137 days) and *ultra-late ripening* (154-158 days); ***semi-determinate:*** *early ripening* (102-109 days), *mid-ripening* (110-117), *late ripening* (121-132 days); ***superdeterminate:*** *ultra-early ripening* (87-98 days), *early ripening* (100-109 days), *mid-ripening* (111-119 days); ***dwarf forms:*** *ultra-early ripening* (79-99 days), *early ripening* (101-109 days), *medium* – (112-119 days) and *late ripening* – (121-147 days). This is a kind of tomato gene pool that determines a wide range of life forms with different types of growth and fruit ripening periods, which can be used as the initial material for these traits when solving theoretical and practical problems of breeding in open and protected ground.

Identification and systematization of mutant forms based on fetal traits. The gene pool we studied is widely represented by marker genes that control various fetal traits [30]. ***Fruit color*** is associated with a large number of mutant genes: *o, at, ep, dg, gf, hp, t, u, ug, Ip, l, r, sh, y,* which determine the nature and intensity of the color of the ripening fruit, and the expression degree of the differences in mature ones. The content of ***lycopene*** and ***β-carotene*** in fruits is controlled by the *β, βc,* β^{og}, and *Del* genes. By changing the biochemical, and especially the pigment composition of fruits, these genes affect not only the color, but also the taste, dietary and commercial qualities of the fruits [1, 43, 55]. Color genes most strongly influence the pigment composition of fruits, while some mutant genes – *r, at, sh* reduce the total content of carotenoids [60], and others – *hp, hp-1, hp-2, Ip, dg* increase it [61], and the third ones – *β, βc, Del, t, gs, fg* change their composition and ratio without affecting the total content. The *β* gene is of great value for improving the chemical composition of fruits, which increases the content of *β*-carotene, the main precursor of vitamin A. Forms with the *β* gene, as a result of the substitution of lycopene with *β*-carotene, have an orange or red-orange color of the fruit [39, 55]. The *pat* and *pat-2* genes give fruits its ***fleshiness and high elasticity***. Their use in breeding improves fruit formation and fruit size, especially when grown in unfavorable conditions. The ***degree of pubescence*** of different parts of the plant, including fruits, is determined by the genes *Ln, p, vi,* W^{om}. Unripe fruits have mutant forms that are carriers of the *rin, nor, alc* genes. The trait – ***the shape of the tomato fruit***, as well as color, is one of the main characteristics in the breeding of new varieties regarding their direction and purpose [1]. The specificity of the collection in terms of fruit shape is given by the presence of another group of genes in it – *Ol, o, obl, el, n*. ***The surface of the fruit*** in the studied forms is smooth or ribbed to varying degrees; the apex of the fruit is smooth, sometimes elongated into a more or less sharp nose. According to the degree of phenotypic expression of these traits, mutant genes have been described that control the pattern of their manifestation, *ep, o, el, crl, f, y, bk, lc, Ol, n, n-2, ck, anr, ptb* [30]. Mutant forms of tomato have been identified and systematized according to ***the number, shape, color, structure of seed chambers, modification of fruit ripening and the presence of seeds in them***, controlled by genes *–an, aw, e, ds, Ge, at, Bog, Lc, Ip, Del, Abg , at, gf, Gr, hp-1, lo, loc, Nr, r, t, fi, bs* [30]. Taking into account the pattern of the manifestation of fetal traits for their targeted use in breeding, they are divided into the following groups:

Group I – round-shaped fruits of varying color intensity (16 varieties according to the color scheme of the fruit): 1 – red; 2 – intense red; 3 – carrot-red; 4 – orange; 5 – yellow and dirty yellow; 6 – pink; 7 – white; 8 – dark green and green-yellow; 9 – intensely green pubescent; 10 – red-burgundy, turning into black; 11 – brown-black; 12 – red with pink stripes; 13 – pink with yellow stripes; 14 – red with yellow stripes; 15 – green with yellow stripes; 16 – orange with pink stripes.

Group II– flattened fruits (divided into three varieties according to the characteristics of the surface of the fruit in combination with the color of the fruit): ***A***– *smooth surface* (1 – bright red, 2 – dark red, 3 – green); ***B*** – *semi-ribbed surface* (1 – red, 2 – intense red); ***C*** – *heavily ribbed* (1 – red, 2 – dark red, 3 – intense pink with orange stripes).

Group III– oblong fruits (divided into five subgroups according to the shape of the fruit): ***A***– *pear-shaped* (1 – red, 2 – lemon yellow, 3 – intense orange, 4 – pink); ***B*** – *plum-shaped* (1 – red, 2 – orange, 3 – banana color); ***C*** – *cylindrical* (1 – red, 2 – orange); ***E*** – *heart-shaped* (red, pink); D – ellipsoidal (1 – red).

This is an inexhaustible source of germplasm, which opens up wide opportunities for their active use in the creation of new varieties and hybrids of tomatoes for various purposes, including ornamental ones.

According to the author of more than 500 mutant forms of tomato [33], for their targeted and effective use in breeding and genetic research, it is necessary to have information about the degree of phenotypic variability of marker traits in the crop cultivation area. Our studies [30] have shown that the manifestation and reliability of identification of mutant genes simultaneously depend on both the characteristics of the genotype and environmental conditions. It has been established that different marker characteristics in unregulated climatic conditions (in the field) manifest themselves ambiguously. The presence of anthocyanin on the hypocotyl of mutants, as well as its content on various parts of the plant (*a, aa, aw, at, al, ag, bls, cla* etc.) are most susceptible to the influence of environmental factors. In some cases, anthocyanin pigment is strongly expressed, in others it is not detected. A similar situation was revealed by the modification of hairs controlled by genes *h, hl, hr, hrt, hs, vi, Ln* etc. To a certain extent, this also applies to mutants with unusual color and shape of the fruit with uneven spotting and necrosis of leaves (*Abg, af , at, B, bk, coa, ctr, cul, dg, alb, v-2, impdia, psu* etc.). A wide range of variability in the nature and degree of phenotypic manifestation was noted for sterility markers (*ex, ex-2, ps, ps-2, s, sl-2, st, ste*) and genes (*mup, mux, mua, ms-2, uf),* controlling the type of inflorescence and the number of flowers on them. Another group of marker genes: *alb, ch, cv, lur, lut, marm, spl, scf*is very well identified at the cotyledon leaf stage and during the growth and development of seedlings in a greenhouse, but after planting the plants in a permanent place in the field, the signs disappear. Many marker traits are identified only at a certain stage of growth and development (seedlings, cotyledons, first true leaves, flowering, full ripening of fruits, etc.), which confirms the author's recommendations about the effectiveness of their use in practical breeding only if information on the degree of their phenotypic manifestation is available.

A comprehensive study of the gene pool of mutant forms of tomato (mutant genes) has shown that they are a unique source of new germplasm, determining a wide range of life forms, which can be purposefully and actively used to solve theoretical and practical breeding problems (creation of new generation initial material, lines, varieties and heterotic hybrids), as well as a tool (object) for conducting fundamental genetic, physiological and biochemical research [30].

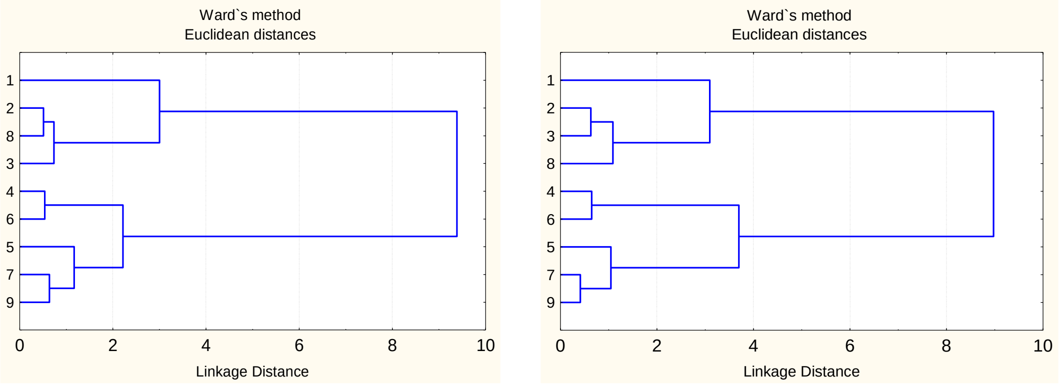
5. GAMETE TECHNOLOGIES AND THEIR USE IN TOMATO BREEDING AND GENETIC RESEARCH

5.1. Variability of morphological traits of the nuclei of vegetative (V) and generative (G) cells of tomato pollen in F_1 lines and hybrids under the influence of stress temperatures. The results of the study of morphological characteristics – diameter, perimeter, area in the nuclei of ***vegetative (V)*** and ***generative (G)*** cells of tomato pollen when exposed to high (45ºC) and low temperatures (+7ºC) in parental lines (P_1 and P_2) and their F_1 hybrids are presented. It has been shown that the nuclei of V and G cells react differently to high and low temperatures. The root cause of their different reactions may be the structure: the nuclei of V cells are vacuous, large, in contrast to the nuclei of G cells, which are denser and smaller. The nuclei of V and G pollen cells differ in homozygous and heterozygous genotypes and in the composition of hereditary factors.

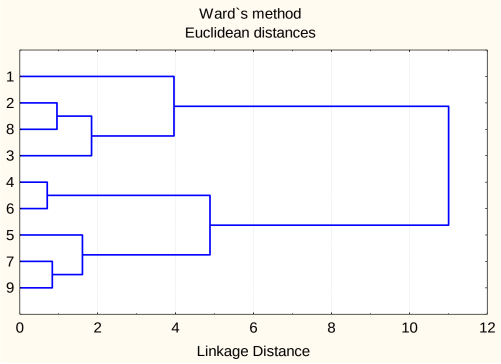
It has been found that the nuclei of V cells in the populations of all F_1 hybrids are more homogeneous in morphological traits than those of their parental forms. And, conversely, in terms of the traits of G cell nuclei, especially in experimental variants, variability is higher in F_1 populations.

Clustering of genotypes according to the pattern of the manifestation of morphological characteristics of the nuclei of V pollen cells at 25ºC (control) revealed the following distribution: the 1st cluster included genotypes with average values of the traits (diameter, perimeter, area of nuclei), the 2nd with low values, and the 3rd with high ones. When pollen is exposed to a temperature of 45ºC, the degree of similarity between genotypes changes, and they end up in different clusters. While a temperature of 7ºC does not have a special effect on the variability of the trait of V cell nuclei, the degree of similarity between genotypes relative to the control almost does not change (Fig. 5). The pattern of the manifestation of the traits of the nuclei of V pollen cells in F_1 is predominantly determined by the paternal form.

Analysis of the morphological characteristics of pollen G cell nuclei on three different backgrounds (25ºC, 45ºC, 7ºC) showed their higher variability. Clusters had pronounced trait indicators (Fig. 6). For example, in the control (25ºC), the magnitude and pattern of the manifestation of traits is determined by the influence of the paternal form in all hybrids, while in the variants with temperatures of 45ºC and 7ºC a high influence of the maternal forms is found. The response of the original forms and F_1 hybrids of tomato according to morphological characteristics to the action of stress factors manifests itself in a differentiated manner. At the same time, genotypes in populations from different combinations based on the traits of G cell nuclei at both backgrounds (45ºC and 7ºC) had a similar distribution into clusters (Fig. 6). It is likely that selection for resistance to one factor may lead to increased tolerance to another.

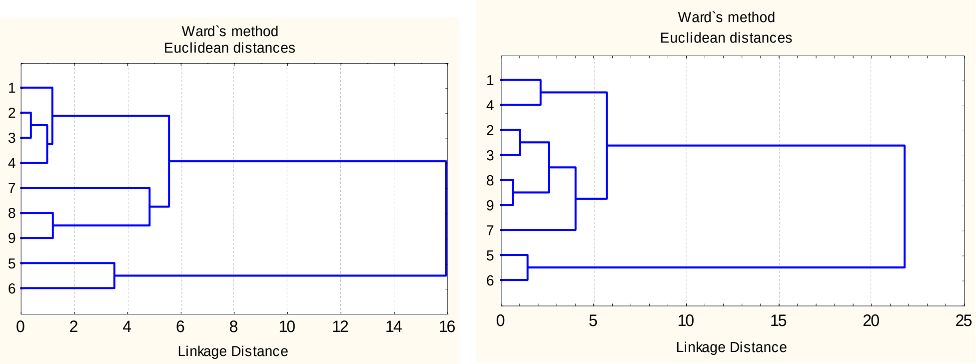


25°C 45°C

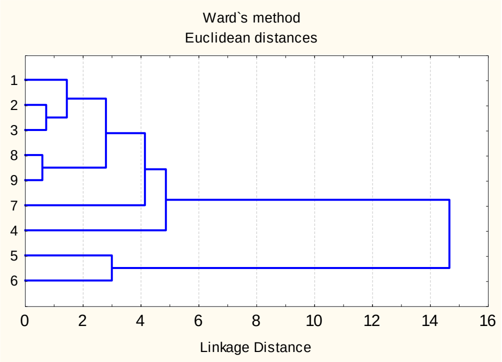


7°C

Fig. 5. Dendrogram of the similarity of the original forms and F₁ hybrids of tomato according to the morphological characteristics of the nuclei of V pollen cells at high and low temperatures: ***1.*** *L 187(1);* ***2.*** *L 965;* ***3.*** *F₁ Irok;* ***4.*** *L 187(2);* ***5.*** *L 828;* ***6.*** *F₁ Krasnaia Strela;* ***7.*** *L 965;* ***8.*** *L 214;* ***9.*** *F₁ Severniy Express*



25 °C 45 °C



7 °C

Fig. 6. Dendrogram of the similarity of the original forms and F₁ hybrids of tomato according to the morphological characteristics of the nuclei of G pollen cells at high and low temperatures: ***1****. L 187(1);* ***2****. L 965;* ***3****. F₁ Irok;* ***4.*** *L187(2);* ***5.*** *L828;* ***6.*** *F₁ Krasnaia Strela;* ***7.*** *L965;* ***8.*** *L214;* ***9.*** *F₁ Severniy Express*

5.2. Variability and inheritance of DNA content and chromatin dispersion.

We studied the influence of high (45ºC) and low (7ºC) temperatures on the pattern of manifestation and variability of cytochemical parameters (DNA content and chromatin dispersion) of the nuclei of vegetative (V) and generative (G) pollen cells of F_1 tomato hybrids and their parental forms (three hybrid combinations).

In terms of DNA content in the nuclei of V and G pollen cells, clear differences were observed between the hybrids and their parental forms. These differences are especially pronounced against the backdrop of stressful temperatures. This may be due to the fact that gametes of heterotic genotypes can be of different types and vary in the composition of hereditary factors. Consequently, they respond differently to stressful temperatures, thereby creating a wider range of variability. Since the nucleus of the G cell is the carrier of hereditary information, this indicates a broad connection between genomic changes, including those associated with hybridization, while the nucleus of the vegetative cell is a storage cell with high metabolic activity.

Significant clear differences were revealed between the parental forms and their F_1 hybrids in the nuclei of both (V) and (G) pollen cells in terms of chromatin dispersion, which characterizes its functional activity. An increase in its index demonstrates a more compact structure and a decrease in its activity [34]. No specific pattern of influence of the used temperature stress factors on the variability of the traits DNA content and chromatin dispersion" has been established. The manifestation of these traits in F1 tomato hybrids was predominantly determined by the genotypic particular features of the parental forms.

A relative index of the thermal resistance of genotypes can be the ratio of DNA content in the nuclei of V and G pollen cells (G/V), which characterizes the process of DNA synthesis [13]. It should be noted here that we obtained pollen from plants grown in a greenhouse, where the temperature in summer exceeded 30ºC, so the G/V ratio in the control actually characterizes its stability, and its subsequent warming created additional stress. It was found that the resistance of pollen of all three F_1 hybrids to the studied temperature stress (45ºC and 7ºC) was either lower than that of the resistant parental form, or coincided with the less resistant form (Table 5). This indicates that the original lines are characterized by different genetic resistance.

Table 5. The ratio of DNA content in the nuclei of G and V pollen cells in the initial lines and F_1 hybrids of tomato under the influence of different temperatures

F_1 hybrid	**t = 25ºC (control)**			**t = 45ºC**			**t = 7ºC**		
	P_1	P_2	F_1	P_1	P_2	F_1	P_1	P_2	F_1
Irok	1.20	1.15	1.09	1.28	1.04	0.96	1.38	1.21	1.19
Krasnaia Strela	1.14	1.09	1.18	1.23	0.91	0.85	1.32	1.08	1.08
SeverniyExpress	1.35	1.25	1.24	1.31	1.21	1.31	1.01	1.22	1.06

This indicates that the original lines are characterized by different genetic resistance. Therefore, during hybridization, in addition to taking into account the resistance indices of the male gametophyte, it is necessary to select initial lines with high combining ability and identical resistance to temperature stress.

At the same time, we analyzed the influence of genotype and temperature factors on the variation in DNA content in the nuclei of V and G pollen cells. It was found that the share of the influence of the interaction of two factors on the variability of the amount of DNA in the treatment with high temperature is significantly higher in the G cell and is 78.4% versus 47.4% in the V cell (Table 6). Against the background of low temperature, both in the V and G cells, the influence of the interaction of factors “genotype x temperature” is quite high and amounts to 62.6% and 60.6%, respectively (Table 6). This indicates reserves for selection of forms resistant to this stress with the desired indices of adaptability.

The share of the influence of genotype, temperature and their interaction on the variability of the chromatin dispersion trait is somewhat different. High variability of chromatin dispersion was noted during the interaction of these factors in the option of using pollen at high temperatures (45°C) for V cell - 66.1%, while for G cell only 38.6% and 23.1% are determined by genotype. Against the background of low temperature, the share of influence of genotype (32.8% V - cell and 32.1% G - cell) and temperature (32.8% V - cell and 44.8% G - cell) is high (Table 6), which led to a clearer differentiation of genotypes. Possibly, pollen resistance is associated with the functional activity of chromatin.

Table 6. The share of the influence of various factors on the DNA content and chromatin dispersion in the nuclei of V and G pollen cells under the influence of temperature stress

Factors	**DNA content**				**Chromatin dispersion**			
	t = 45°C		**t = 7°C**		**t = 45°C**		**t = 7°C**	
	V	**G**	**V**	**G**	**V**	**G**	**V**	**G**
Genotype	16.0	18.3	23.2	29.3	9.0	23.1	32.8	32.1
Temperature	23.4	4.1	8.1	1.8	5.9	9.4	32.8	44.8
Genotype x Temperature	47.4	78.4	62.6	60.6	66.1	38.6	26.6	10.0

.

Thus, the manifestation of cytochemical traits such as DNA content and chromatin dispersion in the nuclei of V and G pollen cells in all studied tomato F_1 hybrids was either intermediate or close to the less resistant parental form, which indicates the recessive pattern of inheritance of resistance to the studied temperature stress factors. Similar results were obtained by other authors [6, 56], who identified genomic changes in F_1 maize hybrids indicating instability of nuclear DNA content. It was also shown that in interline hybrids with high heterosis, the amount of nuclear DNA was at the level of the average parent, while in F_1 with low heterosis, the amount of DNA was approximately 5% higher [37]. High variability of DNA content depending on environmental conditions as beenestablished in allopolyploid hybrids of triticale and wheat [2, 4].

5.3. Inheritance of the resistance to high and low temperatures for male gametophyte traits by F_1 hybrids

We studied the inheritance of resistance according to the traits of the male gametophyte (pollen viability, pollen tube length, resistance to pollen germination and pollen tube length) to high (45ºC) and low (7ºC) temperatures by F_1 hybrids obtained by direct and reciprocal crossings of lines with different resistance types and levels. It has been established that when crossing two resistant lines (L187 x L828) according to the studied traits in F_1 hybrids, both in terms of heat resistance and cold resistance, negative over dominance (depression) is observed in direct and reciprocal hybrids (Table 7), which indicates the recessive nature of these traits.

In the hybrid combination (L187 x L965), where one of the parent components is resistant to both temperatures, and the other is highly sensitive, negative dominance and over dominance in heat and cold resistance of pollen are observed. The same pattern was revealed for the stability of pollen tubes (Table 7). The conclusion about the recessive nature of resistance based on these traits is also valid in this treatment.

In F_1 hybrids, where both parental forms (L558 x L965) are susceptible to high and low temperatures, there is over dominance in pollen heat resistance and pollen tube stability. For the trait "cold resistance of pollen", in direct crossing, intermediate inheritance was observed, in reciprocal crossing, dominance of the best parent was notable (Table 7). The indices are more pronounced against the high temperature background.

It can be assumed that these lines are closely related in origin, the crossing of which leads to the unification of their alleles and increased resistance in F_1.

Direct and reciprocal hybrids F_1 (L469 x L186 and L186 x L479), produced using parental forms with the same average resistance to stressful temperature factors according to male gametophyte traits, form pollen that is more heat-resistant than their original forms. This also applies to the traits cold resistance and stability of pollen tubes. It is likely that the lines (469 and 186) used in this crossing combination also have different pairs of equivalent resistance genes, the combination of which in one genome in F_1 hybrids leads to increased manifestation of traits characterizing the resistance of the genotypes.

Table 7. The pattern of inheritance of resistance to high and low temperatures by F_1 tomato hybrids for the traits of the male gametophyte

Hybrid combination	**The inheritance coefficient, hp**					
	Freshly collected pollen (control)		**Heat resistance**		**Cold resistance**	
	1	**2**	**3**	**4**	**5**	**6**
L187 × L828	- 1.4	+ 2.0	- 0.55	- 1.5	- 6.0	- 7.1
L828 × L187	- 1.6	+ 3.3	- 1.21	- 2.65	- 5.2	- 7.2
L187 × L965	+ 3.9	+ 6.4	- 0.71	- 0.86	- 1.0	- 153.8
L965 × L187	- 1.5	+ 3.7	- 0.82	- 0.30	- 0.70	- 139.6
L558 × L965	- 1.2	- 0.06	+ 16.4	+ 2.2	+ 0.33	- 0.94
L965 × L558	- 5.5	- 0.13	+ 26.9	+ 2.8	+ 1.0	- 1.20
L469 × L186	+ 1.5	+ 0.89	+ 1.43	+ 24.0	+ 4.9	+ 0.06
L186 × L469	+ 0.4	+ 0.56	+ 3.0	+ 79.6	+ 8.3	+ 1.4
L965 × L214	- 1.0	- 0.33	+ 1.1	+ 0.28	+ 41.0	+ 0.42
L214 × L965	- 0.5	- 0.06	- 0.18	+ 3.3	+ 12.8	- 0.68
L189 × L965	- 2.6	+ 0.6	+ 67.7	+ 4.48	+ 0.69	- 3.7
L965 × L189	- 20	- 1.0	+ 49.8	+ 0.40	- 0.94	- 9.4

Note. ***Numbering in the table headings:*** **1**. Viability of freshly collected pollen; **2.** Length of pollen tubes; **3.** Heat resistance for pollen germination; **4.** Heat resistance for the length along pollen tubes; **5.** Cold resistance for pollen germination; **6**. Cold resistance for the length along pollen tubes

There were no significant differences depending on the direction of crosses in F_1. The established patterns of inheritance by F_1 hybrids of resistance to high and low temperatures based on male gametophyte traits when crossing forms with contrasting and equal levels of resistance ***also indicate the recessive nature of the inheritance of traits to stressful temperature factors, also indicate the recessive nature of the inheritance of traits to stressful temperature factors,*** which is consistent with the conclusion made in section 5.2 and the conclusions of other authors [17, 24, 52].

The findings are of great interest for practical employment in tomato breeding and can serve as a prerequisite for a reasonable selection of parental pairs in heterotic breeding.

5.4. The share of the influence of parental forms on the heritability of resistance to temperature stress

The influence of parental forms on the manifestation of heat and cold resistance traits in F_1 was determined by component analysis in a two-factor complex with the calculation of the broad sense heritability coefficient (h^2). Evaluation and analysis of parental forms and their F_1 hybrids (14 combinations) for resistance to high temperatures were carried out at various temperature backgrounds: 35˚C; 38˚C; 45˚C and 48˚C with different time exposures to pollen: 3, 5 and 7 hours. Cold resistance was determined by germinating pollen in vitro at low temperatures of +6˚C and +10˚C. The share of influence of each parent and their interaction on the heritability of resistance to temperature stress factors based on male gametophyte traits was calculated [28].

In general, when pollen was exposed to temperatures of 35˚C, 38˚C, 45˚C and 48˚C with temporary exposures of 5 and 7 hours, the influence of the interaction of maternal and paternal forms (A x B) on the heritability of the trait "heat resistance" by F_1 hybrids was significant pollen" F_1. When assessing each F_1 combination individually, the influence of maternal forms (A) was decisive, especially in the case where a more stable form is used as the maternal component.

It was found that the manifestation of the trait "viability of freshly collected pollen - control" in F_1 is determined by the interaction of maternal and paternal forms (A x B).

Treatment of pollen from F_1 hybrids at a temperature of 35˚C for 5 hours revealed a significant effect on the manifestation of the trait in the F_1 maternal form (55.4%). With a longer time, exposure to pollen (7 hours), these values change. The same pattern is observed at a temperature of 38˚C with a time exposure of 5 hours. A longer exposure to pollen (7 hours) leads to an increase in their interaction (A x B) - 68.0% (Table 8).

Exposure of pollen to a temperature of 45˚C for 5 hours shows that the resistance of F_1 hybrids is mainly determined by the maternal component (52.4%). An increase in the time exposure of the action to 7 hours leads to a decrease in the proportion of the maternal component (A) and an increase in the influence of the interaction of maternal and paternal forms (A x B) on the heritability of pollen heat resistance by F_1 hybrids (Table 8).

A significant influence on the heritability of the trait in F1 at a temperature of 48˚C with different durations of action (5 and 7 hours) is exerted by the interaction of (A × B) factors with coefficients of 61.6% and 71.2%. The share of influence of paternal forms (B) in all treatments of research turned out to be insignificant (Table 8).

Table 8. Heritability of the trait *pollen heat resistance* by F_1 hybrids

Crossing components	**h^2, %**				
	25˚C – 3 hours (control)	**35˚C**	**38˚C**	**45˚C**	**48˚C**
	5 hours				
A♀	12.4	55.4	44.0	52.4	28.6
B♂	16.2	7.5	15.2	9.2	7.2
A × B (♀ × ♂)	66.7	35.1	39.3	37.9	61.6
	7 hours				
A♀	12.4	37.6	21.9	35.9	19.0
B♂	16.2	9.4	10.0	9.5	7.5
A × B (♀ × ♂)	66.7	52.7	68.0	54.4	712
Note: ♀ – L 5 and L 7; ♂ – L 302, L 304, L 305, L 311, L 324, L 327, cultivar Viza.					

The second block shows an analysis of the heritability of the trait "cold resistance of pollen" by F_1 hybrids from different combinations, which demonstrates a high share of the influence of the maternal form (A) – 40.6% against the background of a lower temperature (+6˚C). This figure decreases to 18.0% at a temperature background of +10˚C (Table 9), but at the same time the proportion of interaction between the maternal x paternal form (A x B) is very high. In the studied combinations of F_1 hybrids in both experimental variants (+6˚C and +10˚C), the influence of interaction (A × B) turned out to be significant with the coefficients being 55.7% - 78.8%, relatively (Table 9).

Table 9. Heritability of the trait *cold resistance of pollen* by F_1 hybrids

Crossing components	**h^2, %**		
	25˚ C – 3 hours (control)	**10˚C – 24 hours**	**6˚ C – 24 hours**
A♀	31.6	18.0	40.6
B♂	4.6	3.2	3.6
A × B (♀ × ♂)	62.5	78.8	55.7
Note: ♀ – L 5 and L 7; ♂ – L 302, L 304, L 305, L 311, L 324, L 327, cultivar Viza.			

It was established that in combinations of F_1 hybrids (14 pairs) both in terms of heat resistance and cold resistance of pollen, the share of the influence of paternal forms on the heritability of traits is insignificant. The effect of maternal (A) forms and their interaction with paternal (A x B) forms turned out to be significant. In this experiment, the heritability index is not only a characterization of the studied traits, but also of the artificial background conditions in which the parental forms and their F_1 hybrids were studied.

5.5. The role of transgressions in tomato breeding for the resistance to high temperatures

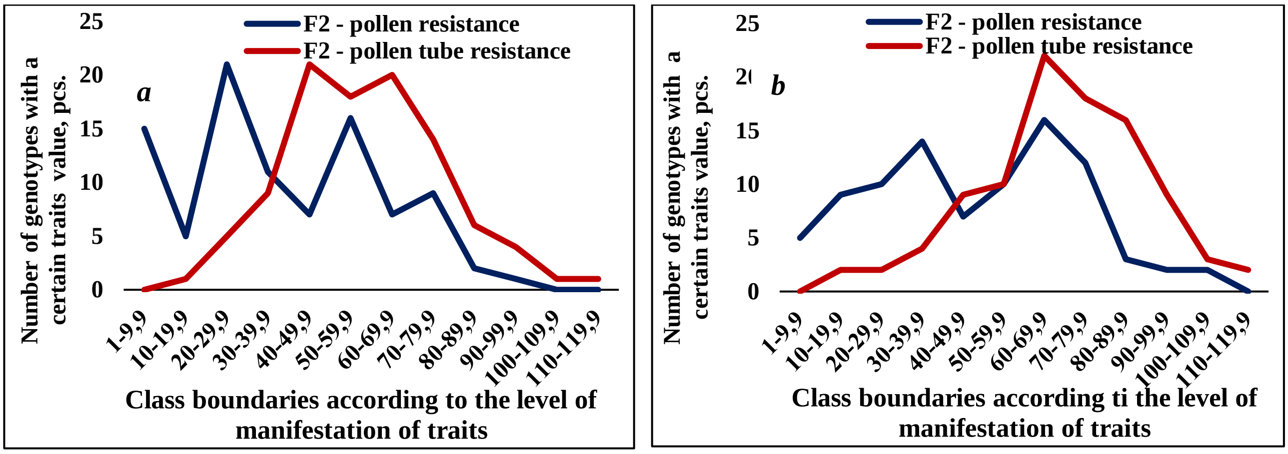
The results of the analysis of splitting F_2 populations (9 combinations) obtained using parental forms that are equally resistant to high temperature (45˚C) according to a set of characteristics of the male gametophyte (pollen viability, pollen tube length, pollen heat resistance and stability along the length of the tubes) are presented. The measure of variation in the studied traits was the average dispersion index (S^2) for the hybrid family [12]. It was shown that the intrapopulation variability in pollen traits for each combination is different: high S^2–8015, S^2–5848, S^2–5764, S^2–5035; average S^2–4645; S^2–4620, S^2–4502 and low S^2–2647; S^2–1702. In the splitting F_2 populations, genotypes with extreme deviations in trait values were identified, namely: inferior to both parental forms; at the level of the mid-parental trait; exceeding or inferior to that of the parents; and transgressive recombinants, which are superior to both parents for pollen heat resistance and tube stability. Figures 7 *a, b, c, d* show the pattern of intrapopulation transgressive variability in pollen traits in hybrids with contrasting dispersion (S^2) in the family.

In the combination of the F_2 hybrid (L5 x L305) with the highest family variance (S^2-8015), a wide range of intrapopulation variability was also revealed. The heat resistance of pollen varies from 1.0 to 89.9%. A high frequency of genotype occurrence (50.8%) with low values of the trait (1.0 - 29.9%) was revealed. The average values (30.0% - 49.9%) were 32.3%, and only 16.9% were characterized by high heat resistance of pollen (50.0 - 89.9%). There are no genotypes in the population with extremely high rates of the trait (90.0% - 109.9%) (Fig. 7 *a*). Analysis of the trait “pollen tube resistance” showed that 53.8% of genotypes were divided into classes with medium and high values (39.9%-69.9%). No genotypes with extremely low values of the trait (0-9.9%) were identified in the population, but a few with extremely high values (100-119.9%) were observed. From this population, only two recombinants were isolated, which had the same high values for both traits, and they were higher than those of the original forms. In the population, many heat resistant genotypes were identified for pollen, but unstable for tube length and vice versa (Fig. 7 *a*).

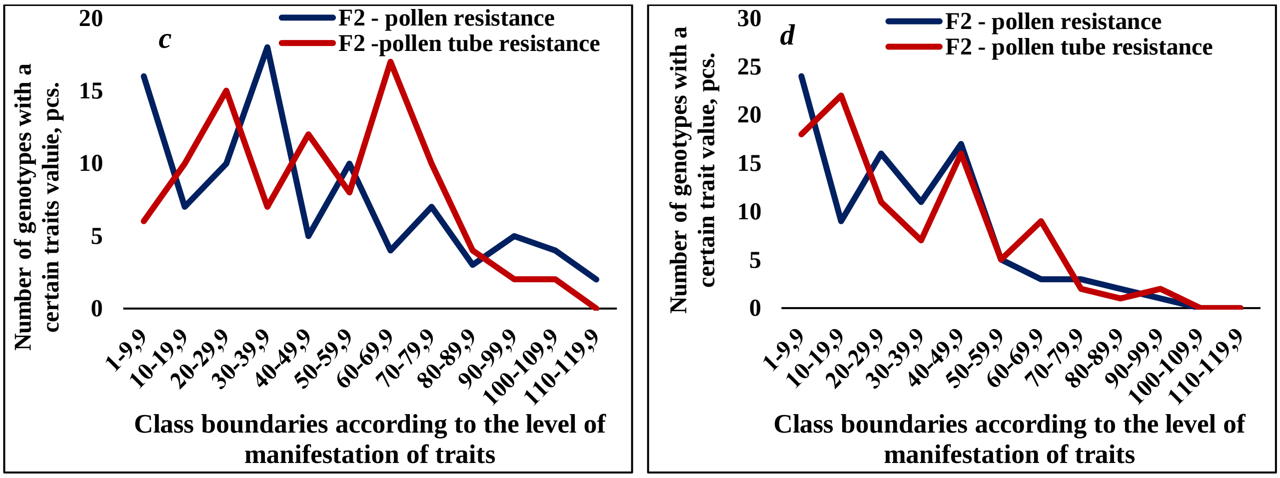
Intrapopulation recombination variability for both pollen traits is quite high and in the combination F_2 – L7 x L126 with dispersion for the family S^2–2647, which is three times lower than in the one discussed above. There are many genotypes in the population (9.2%) with extremely high heat resistance of pollen (from 90.0 to 109.9%), and many recombinants (26.1%) exceeding the best parent in pollen tube resistance (90.0 - 119 ,9%). The diagram (Fig. 7 *b*) clearly shows that the distribution curve of genotypes within the population is shifted towards classes with high values of both traits (50.0% - 89.9%), the frequency of which is 61.2%, and only 12 .7% had low (10-29.9%) and medium (30-49.9%) index scores (Fig. 7 *b*). There are no genotypes with extremely low values (1-9.9%). The occurrence of transgressive recombinant forms with a high coincidence of the values of both traits “pollen heat resistance and pollen tube resistance” in this combination is higher than in the others.

A wide range of intrapopulation variability was also recorded for the L7 x combination Ceros (S^2–5764) for both pollen traits (Fig. 7*c*). Genotypes are represented by all classes: 28.7% with extremely low heat resistance of pollen (1-9.9%); 45.2% had average values (19.9-39.9%); and 23.1% and 2.9% were distributed into classes with high (40 - 89.9%) and very high (90-119%) trait values, respectively. For “pollen tube resistance”, 33.6% of genotypes have extremely low values (from 1 to 19.9%), while the frequency of occurrence of transgressive recombinant forms along the length of the tubes (50-89.9%) makes 41.3%.8.6% of genotypes had extremely high values of the trait (90-119%) (Fig. 7*c*). However, no recombinant forms with a high coincidence of the index values for both traits, which would be higher than that of the best parent, or at its level, were identified.

In terms of the pattern of the variability of traits, the population of the F_2 hybrid – L5 x L324 (S^2–5848) is radically different from those above mentioned (Fig. 7 *d*). A very high frequency of occurrence of genotypes was noted, which were divided into classes with extremely low (1 - 19.9%), low (20 - 39.9%) and average (40 - 49.9%) trait values. But it is from this population that recombinant forms have been isolated, which in both pollen traits exceed the best parent or are at its level. After exposing their pollen to high temperature, it quickly germinates, forming tubes, the resistance of which is higher than in the control (100 - 119.9%). Therefore, the index of this trait should be used as the main criterion in the selection of resistant genotypes.



a **(F_2 – L 7 x L 305)** ***b*** **(F_2 – L 7 x L 126)**



c **(F_2 – L 7 x Ceros)** ***d*** **(F_2 – L 5 x L 324)**

Fig. 7. Genotypic structure of splitting F_2 populations according to the frequency of occurrence of tomato genotypes with a certain value of traits: *heat resistance in pollen germination* and *resistance in the length of pollen tubes*

Individual analysis of intrapopulation recombination variability for the traits *"pollen heat resistance and resistance along the length of tubes"* in combinations with pronounced differences in dispersion (S^2) in the family showed that not all populations produce transgressive recombinants, even if their parental forms are characterized by high resistance. From 9 splitting F_2 populations, it was possible to select 23 genotypes, the genome of which combines genetic factors that ensure, against the background of stressful temperatures, high pollen viability, its resistance, and are also responsible for the growth of pollen tubes and their resistance. Taking into account the identified variability of two pollen traits, it is recommended that selections at this stage of ontogenesis be carried out with a high coincidence of their characteristics.

5.6. Breeding value of F_3 and F_4 generation offspring derived from transgressive recombinants resistant in terms of pollen traits

The response of hybrid populations to selection (R), as one of the most important characteristics of their selection value [9], was determined in F_3 and F_4 by the ratio of the average values of traits of the entire population (X_{avr}) and in selected offspring (X_{op}) in the previous generation. The actual shift in the quantitative expression of the trait during selection was established. Generations were quantified based on the plant selected in the previous generation. Seeds of the best plants isolated in F_2 were used to obtain F_3 populations, and from F_3, the best were selected to form offspring of the F_4 generation.

Analysis of the selection value based on the response of hybrid populations to selection (R) showed that the actual shift in pollen heat resistance in F_3 relative to F_2 is small (0.92), and even lower in F_4 to F_2 – 0.65 (Table 10). Heterogeneity of hybrid populations of different combinations in terms of pollen heat resistance in the F_3 and F_4 generations remains high. Selection was effective in F_3 relative to F_2 only for the combinations L7 x L305 and L7 x Ceros (Table 10), but in F_4 they showed a downward shift in indices. Since the assessment of hybrid populations is carried out in different years, it is obvious that when the conditions for pollen formation changed, not all the best offspring selected in F_2 were such in the next generation, while heat resistant forms were isolated from populations from different combinations. The shift in indices in the dynamics of generations F_2, F_3 and F_4 from different hybrid combinations was slightly higher in the resistance of pollen tubes: 0.99 (F_3 to F_2) and 1.15 (F_4 to F_2). The most pronounced positive shift towards high values for this trait in F_3 and F_4 relative to F_2 was observed in three combinations (R – 1.91, 1.29 and 1.08) (Table 10).

Table 10. Response of hybrid populations F_3, F_4 to selection in F_2 for pollen traits

Hybrid combination	**F_2, % (population average in a specific hybrid combination)**	**The coefficient of selection efficiency in hybrid offspring in generational dynamics**			
		F_3	**F_4**	**F_3 / F_2**	**F_4 / F_2**
	Heat resistance of pollen of hybrid populations in the dynamics of generations				
L 7 × L 305	45.8	1.50	0.86	0.92	0.65
L 7 × L 126	63.7	0.50	0.35		
L 7 × Ceros	53.4	1.11	0.76		
L 5 × L 324	51.5	0.86	0.69		
L 7 × Lider	67.7	0.45	0.37		
L 5 × Ceros	43.3	0.54	0.33		
	Resistance of pollen tube genotypes in hybrid populations				
L 7 × L 305	44.4	1.76	1.91	0.99	1.15
L 7 × L 126	81.1	0.96	0.98		
L 7 × Ceros	64.8	1.16	1.29		
L 5 × L 324	112.4	0.59	0.84		
L 7 × Lider	86.7	0.89	1.08		
L 5 × Ceros	51.2	0.67	0.76		

The homogeneity of these populations in terms of the presence of genotypes with high pollen tube resistance indicates the efficiency of selections taking into account the criterion for this trait in the dynamics of the F_2, F_3 and F_4 generations. The data presented demonstrate that the breeding value of the offspring of the F_3 and F_4 generations obtained from transgressive F_2 recombinants for the traits *pollen heat resistance and pollen tube resistance* is not as pronounced as expected. Probably, the pattern of their manifestation reflects the combination of many genotypic and phenotypic traits that appear at different stages of plant ontogenesis, which simultaneously demonstrates the polygenic nature of the traits.

5.7. Combined selections and their influence on the trait manifestation pattern and intensification of the tomato breeding process.

Combining methods of traditional, classical breeding with gametic selection is aimed at increasing genotypic variability in populations to select highly productive and stable genotypes. A step-by-step alternation of assessment and selection was carried out at different stages of ontogenesis under the influence of high temperatures (43˚C for sporophyte and 45˚C for gametophyte), while recording data on some economically valuable traits (length of the growing season, height of the first inflorescence, early ripening, productivity etc.). Eleven combinations of hybrids were studied and analyzed.

Starting from F_1, the formation and assessment of the offspring of generations up to F_5 was carried out using three methodological approaches: 1 – *step-by-step alternation of sporophytic and gametophytic selections* under the influence of high temperature (**experiment 1**); 2 – *only gametophytic selections* using heat treated pollen (45˚C) for pollination (**experiment 2**); 3 – *traditional selection* during self-pollination for a high rate of studied traits (**control**). Selection index [14] served as criterion, which allows the evaluation of the quality of the lines, the efficiency assessment of the selections and their impact on the intensification of the breeding.

It has been shown that gradual alternation of selections for sporophyte and gametophyte under the influence of high temperature conditions (43°C and 45°C) at the early stages (F_1-F_3) leads to more resistant and productive offspring in F_5-F_7 compared to the use of only gametophyte selections and these differences are more noticeable in comparison to the lines derived through traditional selection (control).

The assessment of F_5-F_7 generation lines at the sporophyte stage (seed germination and seedling length) based on their growth response to high temperature exposure shows that those isolated in alternating sporophytic and gametophytic selections were more resistant compared to the lines isolated using the other two methods. This is especially pronounced for the *seedling length* trait (Fig. 8 *a, b*).

Analysis of the lines isolated from populations of the same combinations, but with different selection methods for male gametophyte traits (pollen germination and tube length), did not reveal a clear influence of the selection method (Fig. 9 *a*, *b*). The most pronounced differences are observed in the traits, as well as in the lines isolated from different hybrid combinations. Pollen from the lines obtained from sporophytic and gametophytic selections was less resistant to high temperature than that from gametophytic selections. However, germinated pollen of the lines from sporophytic and gametophytic selections forms longer tubes (91-214 d.e.m.) than pollen of the lines from gametophytic selections (67-121 d.e.m.). The shortest tubes (44.3-87.5 d.e.m.) were found for the lines in the control treatment. At the same time, it should be noted that according to the traits of the male gametophyte, some lines had equal values, regardless of the selection method used to obtain them (Fig. 9 *a*, *b*).

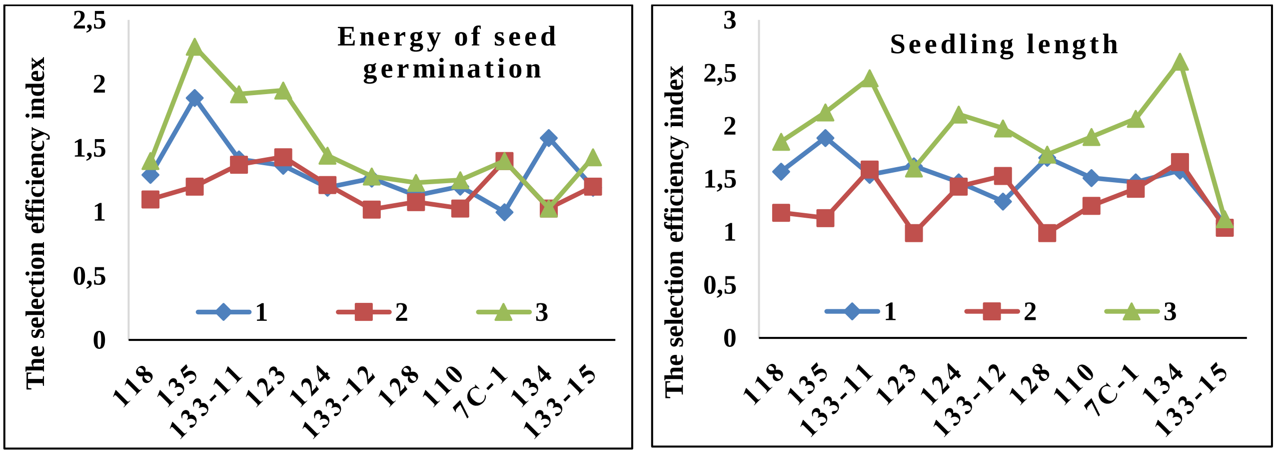


Fig. 8. Selection efficiency index for *seed germination energy* (*a*) and *seedling length* (*b*)

Note: ***1*** *– between sporophytic-gametophytic and only gametophytic selections;* ***2*** *– efficiency index between the gametophytic and control variants;* ***3*** *– alternation of sporophytic-gametophytic selections relative to the control.*

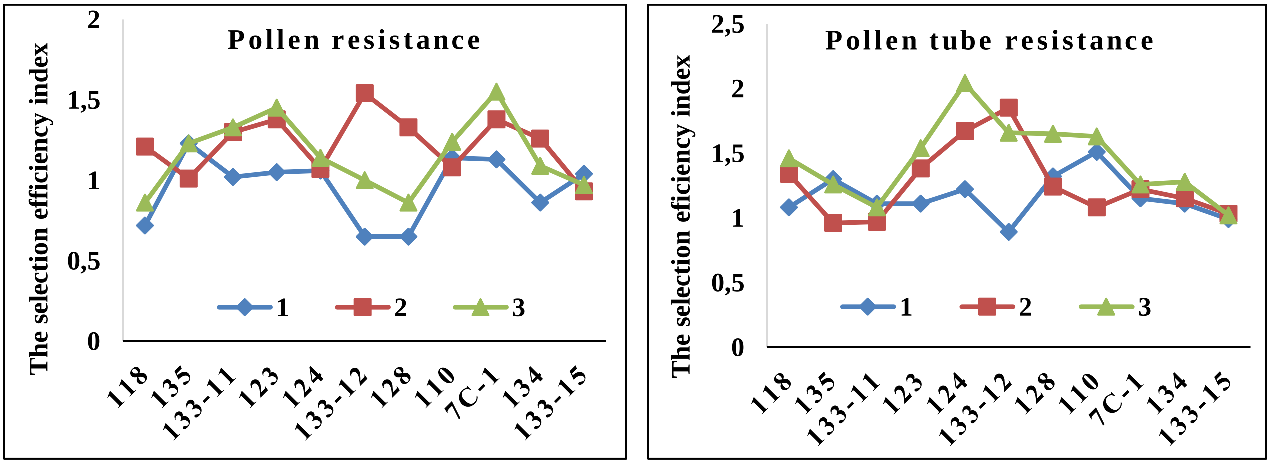


Fig. 9. Selection efficiency indices for *pollen resistance* (*a*) and *pollen tube length* (*b*)

Note: ***1*** *– between sporophytic-gametophytic and only gametophytic selections;* ***2*** *– efficiency index between the gametophytic and control variants;* ***3*** *– indices of alternation of sporophytic-gametophytic selections relative to the control.*

The stable pattern of manifestation and high coincidence of resistance in F_5-F_7 offspring populations in the lines from different combinations against the background of high temperature in the sporophyte (seed germination and length of seedlings) and strong variability in the traits of the male gametophyte (resistance to pollen germination and stability of pollen tubes) in generation dynamics indicates that the role of the diploid generation in the formation of resistance to high temperatures is more pronounced than the haploid generation.

The efficiency of the combined use of traditional selection methods with gamete selection has also been recorded for some economically valuable traits.

In lines 110/1 and 118/1, isolated using alternating sporophytic and gametophytic selections, ***the duration of the growing season*** was shorter than in gametophytic lines by 10 and 11 days, respectively, and by 9 and 8 days compared to the control. In other lines, these differences are not so pronounced (from 1 to 5 days). Analysis of the traits of the ***height of the first inflorescence and the length of the internodes*** showed that the lines 133-11/1, 133-12/1, 124/1, 110/1, and 7/1 from sporophytic and gametophytic selections had a height of the first inflorescence laying by 1- 2 leaves lower than the lines from other selection treatments. These differences are more pronounced in lines 110/1, 118/1 and 124/1, isolated from combinations from crossing ultra-early, early and mid-early forms. The influence of sporophytic and gametophytic selection on the length of internodes was recorded in lines 133-11/1 and 133-12/1, while in the lines isolated from populations of the same combinations, but by two other methods (gametophytic, traditional) such differences were not found.

Data on the ***elements of fruit formation:*** the number of flowers on the first five inflorescences and the number of fruits set from them, depending on the level of the inflorescence position on the plant, revealed a shift in the average values of the traits, both depending on the traits of the parental forms used in crosses and the method of their breeding. The percentage of flower shedding in lines (118/1, 124/1, 110/1, 7/1) from sporophytic and gametophytic selections was lower, and, therefore, fruit formation was higher, which is confirmed by high indices of 1.32; 1.43; 1.21; 1.29 relative to the lines isolated in the control (Table 11). They are also higher and make 1.15; 1.20; 1.13; 1.17 in comparison to the lines from gametophytic selections. The indices are almost equal (1.14; 1.18; 1.07; 1.11) when comparing the lines in the gametophytic selection treatment with the control (Table 11). Probably, for these lines, the direction of alternation of selections at different stages of ontogenesis (sporophyte and gametophyte) against the background of high temperature served as a powerful factor in the development of adaptability associated with genetic and environmental differentiation of lines.

Another group of lines (135/1/2/k, 123/1/2/k, 128/1/2/k and 133-15/1/2/k) decreased fruit formation due to high flower shedding on the upper inflorescences (4-6th). Despite this, there are significant differences between the lines in terms of the selection method used to obtain them (Table 11). Thus, line 135/1 is distinguished by a higher and more uniform fruit set across all inflorescences than those produced from gametophytic (135/2) and traditional (135/k) selections. The results were similar for lines 123/1, 123/2 and 123/k with the indices of 1.14: 1.09: 0.96, respectively. Almost equal indices for all three selection methods for fruit set were found in lines 133-12/1/2/k (1.01: 1.04: 1.03) and 128/1/2/k (1.03: 1 .02: 0.99), while no influence of the selection method on this trait was detected in lines 133-15/1/2 (Table 11).

As for ***overall yield***, the lines isolated from the same combination, but using different methods, also differ greatly from each other (Table 11). Lines 124/1 and 133-

12/1 from sporophytic and gametophytic selections formed a total yield by 9.9 and 7.1 t/ha more than those identified in the control.

Table 11. Fruit formation and overall yield of new tomato lines, derived through different selection methods

Line no.	Flower shedding and fruit formation Efficiency indices of selection methods a:b – a:c – b:c	Total yield selection efficiency indices a:b – a:c – b:c
118/1/2/c	1.15 : 1.32 : 1.14	1.05 : 1.13 : 1.03
135/1/2/c	1.10 : 1.20 : 1.07	1.07 : 1.09 : 0.98
133-11/1/2/c	1.10 : 1.06 : 0.96	1.04 : 1.07 : 1.04
123/1/2/c	1.14 : 1.09 : 0.96	1.07 : 1.06 : 0.99
124/1/2/c	1.20 : 1.43 : 1.18	1.08 : 1.14 : 1.06
133-12/1/2/c	1.01 : 1.04 : 1.03	1.06 : 1.12 : 1.05
128/1/2/c	1.03 : 1.02 : 0.99	1.00 : 1.01 : 1.01
110/1/2/c	1.13 : 1.21 : 1.07	1.09 : 1.11 : 1.03
7-1/1/2/c	1.17 : 1.29 : 1.11	1.04 : 1.05 : 1.01
134/1/2/c	1.13 : 1.12 : 0.99	1.06 : 1.09 : 1.03
133-15/1/2/c	1.03 : 0.94 : 0.92	1.03 : 1.02 : 0.99

***Note:* *experiment-1* (*a* – sporophytic and gametophytic selection); *experiment-2* (*b* – gametophytic selection); *control* (*c* – traditional selection for high values of trait indices)**

The difference in total yield between the lines is 134/1, 133-11/1, 118/1, 7/1, L123/1, and 135/1 in comparison to the control was 4.7 t/ha; 3.9 t/ha; 3.6 t/ha; 2.5 t/ha; 2.4 t/ha, and 2.0 t/ha, respectively. When comparing the total yield between the lines from sporophytic and gametophytic selections and those obtained in the gametophytic treatment only, it is obvious that its index is lower (from 0.3 t/ha to 6.0 t/ha), but it is higher by 0.3 - 3. 9 t/ha than in the control. The indices in the treatment of gametophytic selections are higher compared to the control only in lines 124/2 by 3.9 t/ha, 133-12/2 by 3.1 t/ha, 133-11/2 and 134/2 by 1.4 t/ha, 118/2 by 1.3 t/ha. The differences between the other lines are not so pronounced - from 0.1 to 0.9 t/ha. This is confirmed by the efficiency indices of selection methods (Table 11).

The differences between the lines in the studied traits, both in the reproductive phase during the formation of productivity elements and in the overall yield, give a complete picture of their resistance to high temperatures in field experiments. This confirms the differentiating ability of selection methods.

Evaluation, analysis and selection of genetically different quality breeding valuable material using a combination of traditional breeding methods with gametic selection in the dynamics of F_1-F_7 hybrid generations resulted in complex gene carriers of important traits (more than 100 lines), many of which are used for breeding and genetic research and as donors of economically valuable traits. Others are tested in the control and competitive test nurseries, and some lines have passed the State test. They are approved and included in the Catalog of Plant Varieties of the Republic of Moldova [38].

5.8. Genotypic features of released tomato varieties

General characterization of varieties. An integrated approach to research with simultaneous assessment of resistance to temperature stress and economically valuable traits made it possible to develop new lines and varieties of tomato, *MilOranj; MaKrista; Stefani; Vivat; Prichindel; Cireașca; Matriona; Dimetra, Ilica, Petramak* with an optimal and original combination of morphobiological and economically valuable traits.

Varieties of different ripening periods: early ripening (87-93 days), mid-early (105-110 days) and mid-ripening (112-117 days) are intended for fresh consumption. They differ in the type of plant growth. For example, the *Prichindel* (*dd*) and *Cireașca (ssp)* varieties, which have dwarf and super-determinate growth, belong to decorative and delicious small-fruited (10-18 g) forms of tomato and are intended for growing in loggias and on balconies, as well as in a compacted scheme (8 -10 plant/m2) in open ground. The *Dimetra* variety has a very compact standard type of bush and belongs to the group of ultra-early ripening forms. They successfully combine morphobiological traits with resistance to abiotic stress factors and the most common diseases.

Another group of salad type varieties for lovers are *MilOranj, MaKrista, Stefani, Ilica, Petramak* with super-determinate, determinate and indeterminate growth types. They develop from 4 to 7 inflorescences on the main stem, the first ones alternating through two leaves, and the rest are arranged one after the other, and the growth of the main stem ends in a raceme. the indeterminate, low-growing variety *Stefani* with a low numbers of side shoots and short internodes and the early-ripening determinate variety *Vivat* is particularly distinguished. The fruits of this group of varieties with orange and pink coloration of varying intensity have a presentable appearance and high quality taste. Two red-fruited varieties – *Matriona* (mid-early) and *Mary Gratefully* (mid-ripening) have round, smooth fruits and stable yields. They are highly resistant to low positive temperatures at different stages of ontogenesis (sporophyte-gametophyte) and are suitable for early seedling cultivation.

Resistance of tomato varieties to abiotic stress factors based on pollen characteristics. Targeted breeding for resistance to high, low temperatures and drought allowed us to develop varieties with different combinations and levels of resistance manifestation based on the traits of the male gametophyte, i.e. resistance in case of pollen germination and the length of pollen tubes (Fig. 10 *a, b*). The varieties *MaKrista, Matriona, Ilica, Petramak* are characterized by equally high resistance to all three stress factors for both pollen traits. Pollen of the *MilOranj* variety germinates well against the background of high and low temperatures (47.6% and 48.3%), simultaneously forming long tubes (48-53 d.e.m.). Whereas, against the background of an osmotic stress factor (which imitates drought), the rate of pollen germination is high (63.5%), but germinated pollen grains cannot form tubes of sufficient length for fertilization (12-20 d.e.m.) (Fig. 10 *a, b*).

The variety *Stefani* is characterized by high resistance to pollen germination under different stress backgrounds (54.1% – heat, 77.7% – cold, 63.6% –drought) (Fig. 10 a), but at the same time there are very pronounced differences in the pattern of pollen tube formation. Against the background of osmotic stress, germinated pollen grains cannot form tubes of sufficient length for fertilization, while against the background of high and low temperatures, pollen, when germinating, forms very long tubes – 90 d.e.m. (70.2%) and 79 d.e.m. (77.7%) respectively. Assessment of resistance to low temperature factors (+10ºC) at the sporophyte stage for the trait the length of the embryonic root also revealed high resistance (88.6%). The coincidence of resistance indices at different stages of ontogenesis (sporophyte-gametophyte) is consistent with the high yield when grown in early culture in unheated film greenhouses. The *Stefani* variety also responds positively to cultivation at high temperatures in open and protected ground. The *Vivat* variety is characterized by high resistance to low temperature for both traits (58.0% and 79.8%, respectively), while resistance to drought and high temperature is low for both traits (Fig. 10 *a*, *b*). The reverse reaction of pollen to the action of these stress factors was detected in the varieties *Prichindel* and *Cireaşca.* They are not resistant to low temperatures and are highly resistant to heat and drought. A successful combination of morphobiological and economically valuable traits with stability ensures their stable passage through interphase sub-periods of the growing season and the realization of their genetically inherent productivity potential with high quality fruits under high temperature conditions.

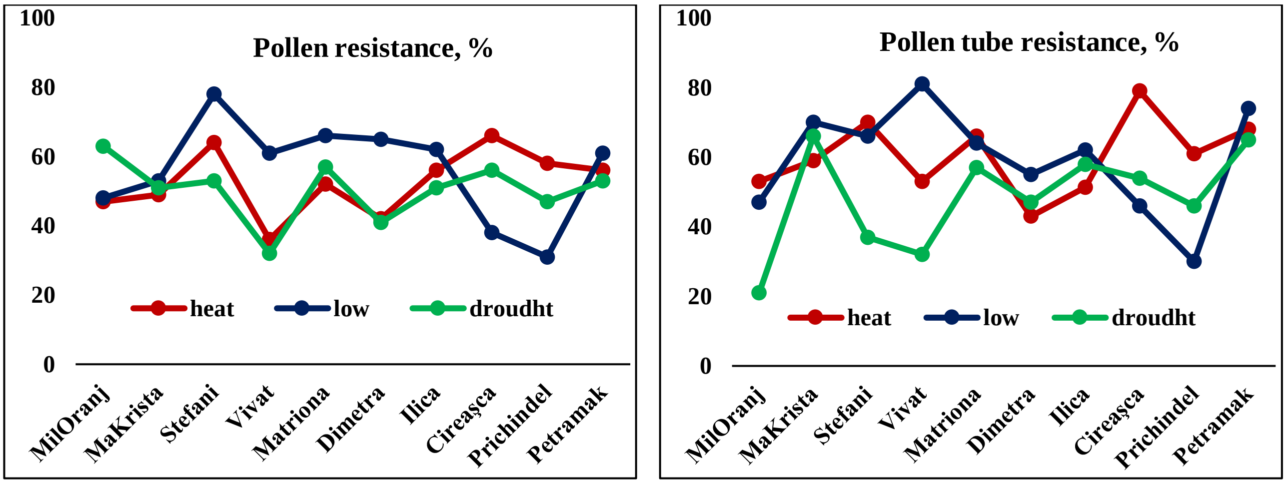


Fig. 10. Resistance of tomato varieties to abiotic stressful factors:
a) *by pollen germination*; b) *for the length of pollen tubes*

Productivity and biochemical characteristics of tomato varieties. Assessment and comparative analysis of new tomato varieties was carried out using the released varieties *Solaris* and *Juliana* as standards for the varieties MilOranj, *MaKrista*, *Stefani* and *Matriona*. For the *Vivat*, *Dimetra* and *Ilica* varieties, the *Peto* variety served as standard. The results obtained for the *Cireaşca* and *Prichindel* varieties were compared with the well-known *Barnaul Canning* variety, which served as a paternal component in the crossing combinations from which the varieties were isolated. Data on recording the total, marketable and early yields of varieties (Xavr) for three years (2015-2017) are summarized in Figure 11.

The *MilOranj* variety had higher yields in three years of different climatic conditions than both standard samples (Fig. 11). The formation of an early harvest, which is equal to that obtained from the first 3 inflorescences, also exceeded their indices. The fruits are dense, large (up to 300 g) with delicate, intensely orange colored pulp with a high content of dry matter (5.78%) and total sugar (4.98%). The *MaKrista* variety is also characterized by high productivity – 66.1 t/ha versus 52.2 t/ha (1st standard) and 58.0 t/ha (2nd standard). The fruits have an excellent taste due to the high content of dry matter (5.2%)

and total sugar (5.0%), intensely pink, large 140-200 g, dense, stays on the plant for a long time (20-25 days) without softening (*nor* gene). Another variety is Stefani with high yields (74 t/ha) and fruit marketability (96.1%), in which almost a third of the yield falls on the first three inflorescences (27.5 t/ha). It is distinguished by large pink fruits (on 1 cluster up to 500-700 g) and long-term yielding time (up to 78 days).

The varieties *Matriona* and *Petramak* also exceed standard samples in terms of general and commercial productivity (Fig. 11). They are characterized by a lower response to environmental factors, forming a consistently high yield in years of different climatic conditions (*Matriona* – 2015 - 67.5 t/ha; 2016 - 65.8 t/ha; 2017 - 66.3 t/ha and *Petramak* – 64.9 t/ha; 68.4 t/ha and 64.3 t/ha, respectively). The fruits of the *Matriona* variety are large (140-200 g), red, with sugary, tender pulp and a high content of dry matter (5.8%) and total sugar (5.04%). The *Petramak* variety also has exceptional characteristics with intensely pink, dense fruits that have high quality taste and contain from 5.0 to 5.7% dry matter and from 4.8 to 5.4% total sugar. The presented varieties are distinguished by high fruit set rate under high temperature conditions and severe drought.

The early ripening varieties *Vivat, Dimetra* and *Ilica* have are distinguished by slightly different characteristics (Fig. 11). They are of a super-determinant growth type. Fruits weighing from 90 to 140 g with high quality taste are different in color – pink, red and orange. Plants with compact inflorescences alternating every 1-2 leaves, with simultaneous ripening of fruits on them and early and synchronous yields.

Varieties – *Prichindel* and *Cireaşca* belong to the group of decorative-gourmet type. They are intended for hobbyists and have high yields, characteristic of the forms of this type (*Prichindel* from 250 to 350 g/plant, *Cireaşcă* from 400 to 750 g/plant). The external presentability of these varieties is ensured by the compact decorative appearance of the plants themselves, the shape of the inflorescences, as well as the shape, weight and color of the fruits. In conditions of high temperatures and drought, the fruits set well.

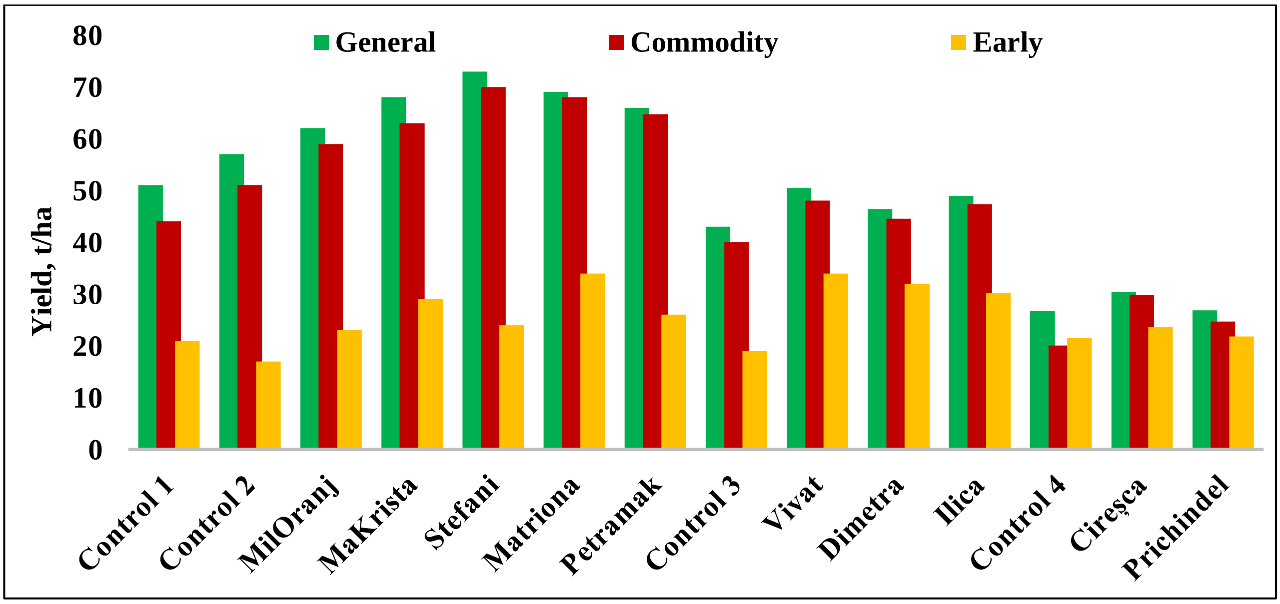


Fig. 11. Productivity of tomato varieties (average for three years with NSR_{05} = 4.03 t/ha for varieties with controls 1 and 2; NSR_{05} = 2.16 t/ha varieties with control 3; NSR_{05} = 1.12 t/ha for the *Cireasca* and *Prichindel* varieties).

The varieties developed are distinguished by their ability to realize the genetically inherent productivity potential with high marketability of fruits in environmental conditions other than optimal. They are characterized by fruits that are uniform in color, shape, weight and color spectrum and have excellent taste qualities. They are recognized and included in the Catalog of

Varieties of the Republic of Moldova [38] (Table 12), and are also protected by patents of the State Agency for Intellectual Property of the Republic of Moldova.

Table 12. Recognized tomato varieties

Variety name	Growing season, days	Fruit weight, g	Regionalization, year	Autors
Mary Gratefully (B)	106-113	80-100	2014	Mihnea N., Grati V., Lupascu G., Botnari V, **Makovey M.**
MilOranj (Br)	113-117	200-300	2014	**Makovei M.,** Guseva L., Beleaev P., Botnari V., Lupașcu G.
MaKrista (Br)	114-119	140-200	2014	**Makovei M.,** Guseva L., Beleaev P., Botnari V.
Stefani (Br)	111-117	200-500	2015	**Makovei M.,** Guseva L., Beleaev P., Botnari V., Ștefîrță A.
Vivat (Br)	90-98	90-120	2016	**Makovei M.,** Guseva L.
Prichindel (Br)	79-85	12-16	2016	**Makovei M.,** Guseva L., Botnari V.
Cireașca (Br)	90-97	10-12	2018	**Makovei M.**
Matriona (Br)	106-111	110-140	2019	**Makovei M.,** Botnari V., Ganea A.
Dimetra (Br)	96-100	80-100	2020	**Makovei M.,** Botnari V.
Iliuca (Br)	104-107	90-110	2020	**Makovei M.**, Botnari V.
PetraMak (Br)	110-115	120-180	2022	**Makovei M.**
F_1 Ingstar	113-117	100-120	In SCZV with 2022	**Makovei M.,** Lupașcu G., Ganea A.
F_1 RozaMak	110-115	110-120	In SCZV with 2022	**Makovei M.,** Lupașcu G., Ganea A.

6. BREEDING FOR HETEROSIS – DEVELOPMENT OF INITIAL MATERIAL

6.1. Prediction of parental form selection efficiency

Currently, the phenomenon of heterosis has acquired enormous industrial significance. The development of heterotic tomato hybrids should be based on the availability of genetically studied material [44] and a continuous screening for donors of such traits as sterility, high reproductive capacity (pollen quality and quantity), organic combination of morphobiological and economically valuable traits, resistance to biotic and abiotic stress factors, early maturity, productivity with high combining ability [7, 10, 16, 20, 29]. The presence of donors with a certain genetic control (mutant genes) and a pronounced phenotypic effect of their manifestation is of particular interest in this regard, the identification of which at the early stages of ontogenesis makes it possible to identify hybrid plants [30]. To predict the efficiency of the selection of parental pairs in the process of creating heterotic F_1 tomato hybrids, we performed hybridization between forms that have no pronounced differences and, conversely, used mutant and semi-mutant forms that differ greatly in the phenotype and genotype (Table 13).

The crossing scheme included semi-mutant lines with a different set of mutant genes: L 8 ($sp^{\pm}$, *u, nor*); L 28 (sp^{+}, *Mi, Tm-2, rin*); L 111 ($sp^{\pm}$, *nor, j-2*); L 1185 (*dw, u, al*); L 135 (*sp, y, br*); L 202 (sp^{+}, *bk, u, ep*); L 11069 (sp^{+}, *nor, br, Tm-2a, ls, j*), direct mutant forms – Mo 409 (*sp, nv*), Mo 443 (*ssp, ls*), Mo 446 (*$sp^{\pm}$, o, r*), Mo 632 (*ag, h, t, l, u, pl, lg*); highly productive varieties of our own breeding – MaKrista, MilOranj, Stefani, and breedings of PNIISKh Zagadka, Fakel, Raiskoe naslajdenie, and Dikaia roza, which are also carriers of the markers *sp, s, u, nor, rin, t, j-2*) (Tab. 13).

Table 13. Scheme for selecting parental forms for crossing at the stage of developing F_1 hybrids and subsequent analysis of the trait inheritance pattern

Plant genotypes of original parental forms		
Hybrid combinations	**Maternal form**	**Paternal form**
L 11069 x L 111	*sp^{+}, u, nor, Tm-2^{a}, ls, br, j-2*	*ssp, nor, u, hp-1, j-2*
L 111 x L 11069	*ssp, nor, u, hp-1, j-2*	*sp^{+}, u, nor, Tm-2^{a}, ls, br, j-2*
L 111 x L 8	- // -	*$sp^{\pm}$, u, nor*
L 8 x L 111	*$sp^{\pm}$, u, nor*	*ssp, nor, u, hp-1, j-2*
L L 8 x L 11069	*$sp^{\pm}$, u, nor*	*sp^{+}, u, nor, Tm-2^{a}, ls, br, j-2*
L 111 x L 28	*ssp, nor, u, hp-1, j-2*	*sp, Mi, g, Tm-2, rin*
L 28 x L 111	*sp, Mi, g, Tm-2, rin*	*ssp, nor, u, hp-1, j-2*
L 111 x Fachel	*ssp, nor, u, hp-1, j-2*	*sp, j-2, u*
L 111 x Makrista	- // -	*sp, j-2, u rin,*
L 111 x MilOranj	- // -	*ssp, u, t, rin*
L 111 x L 1185	- // -	*dw, u*
L 1185 x L 111	*dw, u*	*ssp, nor, u, hp-1, j-2*
L 1185 x Makrista	- // -	*sp, j-2, u rin,*
L 11069 x L 28	*sp^{+}, u, nor, Tm-2^{a}, ls, br, j-2*	*sp^{+}, Mi, Tm-2, rin*
L 11069 x Raiscoe naslajdenie	- // -	*sp^{+}, loc, , u^{+}, s*
L 11069 x Dicaia roza	- // -	*sp^{+}, ep, loc, u^{+}, s*
L 11069 x Stefani	- // -	*sp^{+}, u*
L 111 x Raiscoe naslajdenie	*ssp, nor, u, hp-1, j-2*	*sp^{+}, loc, , u^{+}, s*
L 111 x Dicaia roza	- // -	*sp^{+}, ep, loc, u^{+}, s*
L 111 x Mo 443	*ssp, nor, u, hp-1, j-2*	*ssp, ls*
Mo 443 x Makrista	*ssp, ls*	*sp, j-2, u rin,*
Mo 443 x MilOranj	*ssp, ls*	*ssp, u, t, rin*
Mo 446 x Zagadka	*$sp^{\pm}$,op, u^{+}*	*ssp, u,*
Mo 446 x Raiscoe naslajdenie	- // -	*sp^{+}, loc, , u^{+}, s*
Fakel x Mo 632	*sp, j-2, u*	*ssp, ag, h, t, l, u, pl, lg*
Fakel x L 135	*sp, j-2, u*	*sp, u^{+},j*
L 111 x Mo 409	*ssp, nor, u, hp-1, j-2*	*sp,nv*

Inheritance and the heterotic effect regarding economically valuable traits in F_1 hybrids were studied. It has been shown that the inheritance of the trait ***length of the growing season*** by F_1 hybrids (direct-reciprocal) from crossing the forms where both parents are carriers of the same storability gene *nor* (L11069 x L111; L111 x L8; L111 x L28) followed the type of positive dominance in direct crosses (hp = 1.0-0.87) and over dominance in reciprocal crosses (hp = 1.2-1.8-2.3) (Table 14) with an average heterotic effect for all combinations (16.7%, 120.4%, 105.6%, and 154.3%) (Table 15). Whereas in the combination L 111 × L 28 and vice versa L 28 × L 111, where the parents forms are carriers of two different genes *nor* and *rin*, positive over dominance was recorded (hp = 2.0 - 2.3) in direct and reciprocal hybrids (Table 14). At the same time, a high heterotic effect was observed (168.6% and 208.4%), respectively (Table 15). Hence, two different storability genes present in a heterozygote contribute to delay of fruit ripening more significantly. Similar data were reported by other authors [20, 21].

In combinations with the mutant form Mo 443 – Mo 443 x MaKrista, the duration of the growing season is inherited in F_1 according to the type of positive over dominance (hp = + 2.0), while in Mo443 x MilOranj, according to negative over-dominance (hp = - 1.8) (Table 14), with an average heterotic effect for these combinations of 101.4% and 99.8% (Table 15).

When crossing the mutant form Mo 446 with the early-ripening variety Zagadka, the F_1 hybrid showed intermediate inheritance (hp = + 0.35), while in combination with the late-ripening form (Raiskoe naslajdenie), it showed negative incomplete dominance (hp = - 0.56) of the parent with a longer growing season (Table 14).

The hybrid combination of the Fakel variety with the multi-marker mutant form Mo 632 is of particular interest, where in three different years of research (2014, 2015, 2016), stable negative over dominance was observed (hp = - 3.1 – - 4.2 – - 3.8, respectively), that is, of the parent form with a shorter growing season. Negative heterosis was also recorded for almost all traits (87.1%, 52.2%, 90.7%, and 90.4%), respectively, and for pericarp thickness it made 100.3% (Table 15).

The trait ***length of the growing season*** is inherited by F_1 hybrids from intermediate with deviations towards negative or positive to over dominance, both towards positive and negative (Table 14).

The data and analysis of the pattern of manifestation and inheritance of the trait ***number of flowers per inflorescence*** showed that the degree of dominance (hp) varies widely – from negative overdominance to positive dominance (Table 14). Intermediate inheritance of the trait was observed in 61.2% of F1 hybrid combinations. Five F_1 hybrids had negative over dominance (L11069 x Dikaia rosa, L111 x Raiskoe naslajdenie, L11069 x Stefani, L111 x Dikaia roza, and Fakel x Mo 632). These are hybrid combinations, where one of the initial forms has two-, sometimes three-axis inflorescences, which are inherited according to the dominant type (Table 14). Since the yield depends rather on the ***number of fruits set on the inflorescence*** and their average weight than on the number of flowers, an individual analysis of these traits when growing F_1 hybrids and their parental forms for 3 years (2014-2016) made it possible to identify the peculiarities of their manifestation. For example, in hybrid combinations of L111 x L28 and L28 x L111, L111 x MilOranj, and Mo446 x Zagadka, positive dominance is observed (hp = 0.95 – 0.64 – 0.85 and 0.55, respectively), and positive over dominance in the combination L111 x MaKrista (hp = 1.05) (Table 14). For these hybrids, the average effect of heterosis over the years of research is quite high and amounts to 107.8%, 131.6%, 121% and 126.3%, respectively (Table 15). Consistently high heterosis is also observed in other traits. Intermediate inheritance of the ***trait number of fruits per inflorescence*** was found in 62.0% of combinations. Negative over-dominance occurred only in the F_1 hybrid with the participation of the mutant form Mo 632 (hp = -1.86) (Table 14). Analysis of the ***fetal weight*** trait showed that 33.2% of F_1 hybrids showed positive over-dominance (Table 14), while the rest had intermediate inheritance. The F_1 hybrid Fakel x Mo 632 is the exception since negative over-dominance (hp = -2.38) in fruit weight and negative dominance (hp = -0.51) in pericarp thickness were observed. Most of the hybrids (63.0%) inherited the trait ***pericarp***

thickness according to the type of positive dominance and over-dominance; in the remaining 37.0%, it was inherited intermediately (Table 14).

The highest values for pericarp thickness (0.60-0.90 cm) were found in F_1 hybrids – L111 x MilOranj, L111 x MaKrista, L111 x L28 and L28 x L111, and L 111 x L11069. Negative dominance and over-dominance were more frequently observed in combinations where a non-mutant form with a semi-complex inflorescence and very large fruits is used as one of the parent forms (L11069 x Raiskoe naslajdenie, L11069 x Dikaia roza, and L11069 x Stefani). The traits are inherited in combinations with the mutant forms Mo 409, Mo 443 and Mo 446 from negative over-dominance (depression) to positive dominance, this is especially obvious in the combination with the multi-marker mutant Mo 632 (Table 14).

Table 14. Inheritance of breeding valuable traits by F_1 tomato hybrids

Hybrid combination	**Degree of dominance h_p (average for 2014-2016)**				
	Traits				
	Duration growing season	**Number of flowers per inflorescence**	**Quantity fruits for inflorescences**	**Weight one fetus**	**Thickness pericarp fetus**
L 11069 x L 111	+ 0.6	+ 0.2	+ 0.45	+ 2.0	+ 1.8
L111 x L 11069	+ 1.2	- 0.35	- 0.17	+ 2.4	+ 3.2
L 111 x L 8	+ 0.54	+ 0.62	+ 0.21	- 0.81	+ 0.72
L 8 x L 111	+ 1.8	+ 0.22	+ 0.02	- 0.68	+ 0.57
L 111 x L 28	+ 2.0	+ 0.13	+ 0.95	+ 1.4	+ 4.1
L 28 x L 111	+ 2.3	+ 0.24	+ 0.64	+ 1.7	+ 3.5
L 111 x Fakel	+ 0.56	+ 0.15	+ 0.20	+ 0.60	+ 0.38
L 111 x Makrista	- 1.32	+ 0.40	+ 1.05	+ 1.40	+ 1.12
L 111 x MilOranj	- 1.0	+ 0.25	+ 0.85	+ 2.1	+ 7.4
L 111 x L 1185	+ 0.64	+ 0.11	+ 0.48	- 0.32	- 0.41
L 11069 x R.N.	- 0.81	- 0.50	- 0.14	- 0.61	- 0.57
L 11069 x D.R.	- 0.9	- 1.38	- 0.21	- 0.80	- 0.33
L 11069 x Stefani	+ 0.42	- 1.1	- 0.63	-. 0.27	- 0.61
L 111 x R.N.	+ 0.5	- 1.31	- 0.04	- 0.74	+ 0.12
L 111 x D.R.	+ 0.64	- 4.0	- 0.42	- 0.25	+ 0.03
Mo 443 x MaKrista	+ 2.0	- 0.5	+ 0.27	- 0.40	- 0.28
Mo 443 x MilOranj	- 1.8	- 0.28	+ 0.4	- 0.25	- 0.54
Mo 446 x Zagadka	+ 0.35	+ 0.12	+ 0.55	- 0.40	+ 0.14
Mo 446 x R.N.	- 0.56	- 0.86	+ 0.07	+ 0.53	+ 1.0
Fakel x Mo 632	- 3.70	- 3.0	- 1.86	- 2.38	- 0.51
L 111 x Mo 443	+ 1.8	+ 0.23	+ 0.17	+ 0.50	+ 0.02
L 111 x Mo 409	+ 0.53	- 0.47	- 0.4	+ 0.48	- 0.51

Stable positive dominance and over dominance of the traits over the years of research was observed in F_1 hybrids with semi-mutant lines: L11069 x L111 and vice versa L111 x L11069, L111 x L28, L111 x MaKrista and L111 x MilOranj. These F_1 hybrids have a high heterotic effect for all traits (Table 15).

A pronounced negative over-dominance (depression) was observed for the breeding valuable traits in the F_1 hybrid Fakel x Mo 632: hp = -3.7 (***length of the growing season)***; hp = -3.0 (***number of flowers per plant)***; h_p = -1.86 (***number of fruits set)***; h_p = -2.38 (***average fruit weight***), and hp = - 0.51 (***pericarp thickness***)

(Table 14). Depression for the manifestation pattern of these traits was consistently expressed in three different years of research. This may be due to the pleiotropic influence of a higher number of mutant genes (*ag, h, t, l, u, pl,* and *lg*), which carries the mutant form Mo 632.

Analysis of the manifestation of productivity traits in F_1 showed that in the combinations L11069 x L111, L111 x L8, L111x L28, L111 x MaKrista, L111 x MilOranj, Mo 446 x Zagadka, and L111 x Mo 409, increased productivity (heterosis) was achieved by *increasing the fruit number on the plant and their average weight.* In combinations L111 x L11069 and Mo 446 x Raiskoe naslajdenie, it occurred due to an increase only in the *average fruit weight,* and in F_1–L111 × L1185 and Mo 443 x MilOranj due to an increase *in the fruit number from the flowers number*. They are recommended for cultivation in open and protected soil in the Republic of Moldova.

Table 15. Heterotic effect (X) for breeding valuable traits in F_1 hybrids

Hybrid combination	**Heterotic effect (X, % – average for 2014-2016)**				
	Growing season duration	**Flower number per inflorescence**	**Fruit number per inflorescences**	**Single fetus weight**	**Fetus pericarp thickness**
L 11069 x L 111	116.7	100.1	124.5	204.1	156.7
L 111 x L 11069	120.4	92.4	97.3	223.7	204.6
L 111 x L 8	105.6	112.7	103.3	98.4	104.2
L 8 x L 111	154.3	109.4	100.2	92.3	103.8
L 111 x L 28	168.6	103.1	107.1	138.6	166.7
L 28 x L 111	208.4	110.2	131.6	141.4	164.8
L 111 x Fakel	98.6	94.1	102.4	112.3	93.7
L 111 x MaKrista	96.1	131.2	126.3	140.7	116.2
L 111 xMilOranj	99.1	113.3	121.0	186.8	226.3
L 111 x L 1185	103.2	99.7	107.2	96.2	90.1
L 11069 x R.N.	93.1	84.3	90.0	93.5	96.2
L 11069 x D.R.	100.2	68.7	86.3	91.4	98.0
L 11069 x Stefani	96.4	100.1	97.8	92.7	101.2
L 111 x R.N.	100.3	90.4	98.2	96.7	100.4
L 111 x D.R.	102.0	77.2	88.4	96.6	99.7
Mo 443 x MaKrista	101.4	80.2	93.8	95.2	94.2
Mo 443 x MilOranj	99.2	94.7	100.4	90.1	98.8
Mo 446 x Zagadka	101.1	104.5	109.7	88.2	96.3
Mo 446 x R.N.	92.3	81.4	96.3	102.7	102.0
Fakel x Mo 632	87.1	52.2	90.7	90.4	93.6
L 111 x Mo 443	132.4	102.3	100.4	96.7	100.3
L 111 x Mo 409	111.2	69.8	77.0	100.3	102.4

Other combinations were also identified (L11069 x Raiskoe naslajdenie, L11069 x Dikaia roza, and L11069 x Stefani), in which negative heterosis was recorded for productivity components. It can be assumed that the negative effect occurs due to the *linkage* of genes (*sp*$^+$*, u, nor, Tm-2*^{a}*, ls, br*), the carrier of which line 11069 is, with late ripening genes and the semi-complex inflorescence of the second components of these hybrids. The assumption [21] that late ripening genes have a depressing effect on precocity, overall productivity, fruit weight, smooth maturation and other economically valuable traits is supported by individual F_1 hybrids.

It has been shown that the degree of dominance and the level of heterotic effect in different F_1 combinations for the breeding valuable traits is determined by the genetic particular features of the parental forms used in crosses and the specific interaction of their genomes.

6.2. Development of initial material with functional sterility

Forms with different types of sterility such as Mikado (*ps-positional sterile*), L1751 (*pis-pistilate*), L1132 (*ms* 10^{35} - *male sterile*) with a marker potato leaf (*c*), L 708/69 (*ps -2*) of a standard bush type (*d*), as well as lines 1151 and 11069 which are characterized by partial sterility (17.8% - 11.2%) and are carriers of the *ls* (*lateral suppressor*) and *br*(*brachytic*) genes were used in the second block of crosses. With their participation, 8 hybrid combinations were derived L1132 × L708/69; L1132 × L 1751; Mikado × L708/69; L1751 × Mikado; L1151 × L708/69; L11069 × L708/69; L708/69 × L1751; and L11069 × L1751. When analyzing the splitting F_2 and F_3 populations, the manifestation degree of the reproductive system traits was taken into account the following: type of inflorescence, type of flowering, type of flower sterility (position of the pistil relative to the stamen column), flower size and lobe number. At the same time, the degree of expression of economically valuable traits was studied, i.e. the duration of the growing season, the type of growth and habitus of plants, the size, shape, and color of the fruit.

The range of genotypic variability in F_2 populations according to the manifestation pattern of the traits that determine the type of sterility. In splitting F_2 populations from different crossing combinations, high intrapopulation heterogeneity was revealed for the traits of *inflorescence type* and *flower structure*. Two classes of genotypes have been identified - with simple (5-9 flowers) and complex inflorescences (11 - 40 flowers). According to the pattern of the position of the column relative to the stamen column, the number of perianth elements and androecium, a wide range of variability was revealed within each combination (Table 16).

High recombination variability according to the type of sterility was observed in the population L1132 × L708/69, from which 11 plants emerged. Three of them had a complex inflorescence with 100% sterility of flowers, the other 8 plants were characterized by a simple inflorescence in combination with different types of sterility, but only 5 of them corresponded to the task in terms of the pattern of the pistil position relative to the stamen column (Table 16). Most of the flowers studied (36.5%) had varying degrees of deformed pale stamens with a small amount of shriveled pollen. Along with them, 16.3% of flowers with a short pistil and a deformed stamen column with sterile pollen were described, 19.4% of flowers had a highly protruding long pistil and highly viable pollen, and only 4.0% were distinguished by the complete absence of a stamen column (*sl*) or had single stamens glued to the pistil. The remaining 23.8% of flowers were fertile with highly viable pollen (58.3%) (Table 16). In the splitting F_2 population of this combination, high heterogeneity in the type of plant growth was revealed: standard (SFM), indeterminate and determinate with potato leaf (SFM), determinate with potato leaf and normally developed flowers, highly productive with uniform fruits, as well as forms with potato leaf, complex multi-branched inflorescence, small pale yellow flowers with a highly protruding pistil, sterile pollen and yellow fruits (a rare combination).

In the splitting population from the combination L1751 × variety Mikado, the spectrum of variability in the presence of sterile forms of different types is significantly narrower. Seven plants with a uniform raceme, flowers without deformations, and a pistil protruding from the bud at the lemon-yellow stage, that is, before flowering, were identified. The majority (71.2%) of the flowers had a highly protruding pistil (2-3 mm) (Table 16). This type of sterility is most interesting for heterotic breeding since self-pollination does not occur due to the non-dehiscence of the anthers. There is an opinion [31] that spontaneous cracking of the anthers of such flowers occurs at temperatures of 35ºC and above. High variability in the ratio of long- and short-columnar flowers in plants was revealed in the population L1751 × L708/69. There were 53% of long-columnar flowers with a normally developed staminate column (functional sterility) in the population. A long pistil, but short glued stamens were observed in 14.8% of flowers and 16.5% of flowers had a short style with varying degrees of deformation of the stamens (Table 16).

Table 16. Segregation in populations of F_2 hybrids according to the manifestation of sterility of different types

F_2 hybrid combinations	**Number of studied flowers, (*pcs*)**	**Flowers on a plant with different types of column position, %**				
		Short pistil, high stamen column position, viable pollen	**Long pistil, highly viable pollen (*ps*)**	**Long pestle, deformed and glued pale yellow stamens (*ex-2*)**	**Short pistil, deformed stamen column (*ex*)**	**Long pistil, stamens glued to the pistil with regrowth or complete absence of stamens (*pis*)**
L 1132 × L 708/69	252	23.8	19.4	36.5	16.3	4.0
L 1751 × Mikado	220	20.3	71.2	0	3.1	5.4
L 1751 × L 708/69	200	15.7	53.0	14.8	16.5	0
L 11069 × L 708/69	159	62.7	25.8	7.2	6.3	0
L 1151 × L 708/69	194	62.9	17.5	19.6	0	0

Five plants were identified with slight variability in flower size and position of the style relative to the staminate column within one inflorescence and more inflorescences depending on the tier of their position on the main stem of the plant. In hybrid combinations (L11069 × L 708/69 and L 1151 × L 708/69), where lines with partial sterility (11.2-17.8%) were used as the maternal form, the formative process by type and degree of manifestation of sterility was much narrower. Splitting into fertile, semi-sterile and single highly sterile plants occurred in F_2 (Table 16). The data show a complex pattern of the inheritance of flower components, indicating the need for breeding of forms with a certain combination of style and stamen lengths. Forms in which the pistil has a moderate length in combination with a short stamen column present particular interest for breeding.

The next stage is to study the ***degree of expression of sterility traits in the offspring of the F_3 generation*** derived from plants isolated in F_2. The level of sterility was assessed by counting the number of fruit set from free pollination [31]. A strong split was noted in the F_3 populations from plants isolated in F_2 from the combination L 1132 × L 708/69. At the same time, F_3 populations formed from two other plants (17/16 and 18/16), isolated from the same hybrid combination, showed a high degree of sterility, regardless of the tier of the inflorescence position on the plant and the flowers in the inflorescences (Table 17). A high degree of sterility was observed in F_3 populations from plants selected in F_2 from the combinations L1751 × Mikado and L1751 × L 708/69, where lines with the optimal combination of pistil and stamen lengths were selected in F_3. From the combination L1751 × Mikado, two lines 27/16 and 33/16 were isolated, the level of sterility of which is almost 100% for all inflorescences. Other lines, 44/16, 45/16 were obtained from F_3 from the combination L1751 × L708/69 (Table 17).

A total of 8 promising lines were identified, which in two different years showed a high level of sterility for the percentage of fruit set from free pollination of 86 - 99%. Five lines (18/6, 27/16, 33/16, 44/16, and 45/16) had functional sterility, the other three (9/16, 11/16, 17/16) were characterized by even inflorescences with long-columnar flowers and different degree of deformation of individual stamens and the entire stamen column.

Table 17. Fruit set in sterile forms of tomato in F_3 populations, depending on the level of location of the inflorescence on the main stem of the plant

Genotype No	Percentage of fruit set depending on the tier arrangement of inflorescence during free pollination					
	Inflorescences					
	1st	2nd	3rd	4th	5th	6th
9/16 (L 1132 × L 708/69)	0.02	0.01	0.02	0.05	0.04	-
11/16 (L 1132 × L 708/69)	0	0.02	0.02	0.04	0.03	-
17/16 (L 1132 × L 708/69)	0	0	0.01	0	0	-
18/16 (L 1132 × L 708/69)	0.01	0	0.01	0.04	0	-
27/16 (L 1751 × Микадо)	0	0	0	0.01	0	-
33/16 (L 1751 × Микадо)	0	0	0	0.01	0.01	0
44/16 (L 1751 × L 708/69)	0.01	0.01	0	0.02	0.02	0
45/16 (L 1751 × L708/69)	0	0	0	0.01	0.02	0.03

Morphobiological characteristics of sterile lines of the F_4 generation. When selecting sterile forms, special attention was paid to the presence of complementary characteristics: type of growth, pattern of stem formation, length of internodes and fruit characteristics. The highlighted lines had a simple inflorescence type. L 11/16 and L 18/16 had a minimum number of flowers on an inflorescence (from 3 to 5), L 33/16 inflorescences had the highest (7.5 pieces) (Table 18), but their number can vary depending on the level of inflorescence position on a plant. For example, in L

33/16, as the level of inflorescence on the plant increases, their branching with the presence of a larger number of flowers is observed, in other lines the opposite happens (9/16 and 45/16). Some lines (9/16, 11/16, 17/16/ 18/16, 27/16/ 44/16) have a determinate type of growth with a main stem height of 59 to 90 cm (Table 18), others (33 /16 and 45/16) belong to semi determinate forms. In L 33/16, most of the plants (53%) ceased growing after the formation of 5-6 inflorescences. Along with them, there are plants (11.7%) that completed the growth of the main stem at earlier stages (4-5 inflorescences); their further growth continued due to side shoots. Similar results were obtained for L 45/16, in which 50% of plants ceased growing after the formation of 5-6 inflorescences, which indicates the influence of environmental conditions on the manifestation of the semiderminate type of growth trait.

Table 18. Morphobiological characterization of F_4 generation tomato lines with different types of sterility (average 2014-2016)

Lines	Vegetation period length (days)	Inflorescence number per stem	Flower number per inflorescence	Leaf number between inflorescences	Plant height (cm)	Fetus weight (g)
9/16	107 ± 1.4	4 - 5	4 - 5	2.1 - 1.4	70 - 80	68
11/16	104 ± 0.9	4	3 - 5	2.0 - 1.4	65 - 86	80
17/16	106 ± 1.2	3 - 4	4 - 5	2.0 - 1.0	59 - 81	75
18/16	106 ± 1,7	3 - 4	4 - 5	2.2 - 1.2	60 - 76	65
27/16	117 ± 1.9	4 - 5	5 - 7	2.0 - 1.5	81 - 90	120
33/16	115 ± 1.1	5 - 7	5 - 7 - 9	2.5 - 1.8	95 - 110	125
44/16	102 ± 1.3	5 - 6	5 - 7	2.1 - 1.5	71 - 90	110
45/16	104 ±2.1	6-8	5 - 9	2.4 - 2.0	89 - 118	98

The lines also differ in the traits of the fruit: some (9/16, 11/16, 17/16, 18/16) are red, smooth, flat-round, round-oval in shape, medium in size and medium-early ripening; others (27/16 and 33/16) have large, intensely pink fruits; the third ones (44/16 and 45/16) have intensely red, round-shaped fruits (Table 18). These lines with a favorable combination of economically valuable traits and different types of sterility are of particular interest for heterotic breeding.

6.3. Mutant *ls* and *br* genes and development of genetic variability in F_2 and F_3 hybrid populations

To develop heterotic hybrids with low side shoot forming ability and short internodes, capable of effectively using not only the total area of cultivation structures, but also the vertical one, we included genotypes in the selection process (L 11069 –*sp*$^+$, *ls, br, j-2, Tm*^{2a} and Mo 443 – *ssp, ls*), which are carriers of the *ls* (*lateral suppressor*) and *br* (*brachytic*) genes that control the intensity of the formation of side shoots and the length of internodes. Using these forms and lines (L28, L111, L187, L828, and L556) that have a set of valuable quantitative and qualitative traits with different shoot forming abilities, possessing genes *sp+, sp±, sp, ssp, u, nor, rin,* and *j-2* , and highly productive, large-fruited varieties MilOranj, MaKrista, Fakel, as a result of the crossings, a series of experimental hybrid combinations were developed (L11069 × L111, L11069 × L828, L11069 × L187, L11069 × L28, L11069 × L556, Mo443 × MaKrista, Mo443 × MilOranj, and Mo443 × Fakel.

Analysis of the first generation hybrids (F_1) revealed 100% dominance of the shoot forming ability of the cultivated parental forms. The size of the side shoots varied and was directly dependent on the degree of their development in the cultivated parental forms. The trait ***length of internodes*** was inherited according to the intermediate type. Differences between F_1 hybrids in terms of reproductive system traits were directly dependent on the genotypic particular features of the lines involved in the crosses.

Recombination variability in splitting mutant linear and mutant varietal populations of F_2 for the traits controlled by the ls and br genes. We studied segregating F2 populations from different hybrid combinations (L11069 x L187; L11069 x L828; L11069 x L556; L11069 x L28; and L11069 x L111), the first group derived from crossing indeterminate and semi-determinate forms and the second group includes Mo443 x MaKrista, Mo443 x MilOranj, and Mo443 x Fakel from crossing determinate forms. Four traits were analyzed: the intensity of the formation of side shoots; length of internodes; morphological disturbances in the flower structure and fruit weight (150 plants in each combination). Taking into account the pattern and degree of manifestation within each characteristic, several indices were considered:

– *Intensity of formation of side shoots:* 1 – high degree (from 100 to 71%, when a side shoot is formed at each node or every other node), 2 – medium (from 70-40%, every 2-3 nodes), 3 – low (40-10%, one side shoot per 4 nodes) and 4 – very low (<10%, no side shoots or single reduced ones);

– *Length of internodes*: 1 – long internodes (from 9.1 cm to 12.5 cm and above), 2 – internodes of medium length (from 6.1 cm to 9.0 cm), 3 – short (<6.0 cm);

– *Morphological disturbances in the structure of the flower* (the overall structure of the flower, its individual elements, including heterostyly): 1 – high degree (from 100 to 70%), 2 – medium (70-40%), 3 – low, (40-10 %), 4 – normally developed flowers (<10%);

– *Fruit weight:* 1 – from medium to large (120-71 g), 2 – from medium to small (70-40 g), 3 – from small to very small (40-10 g).

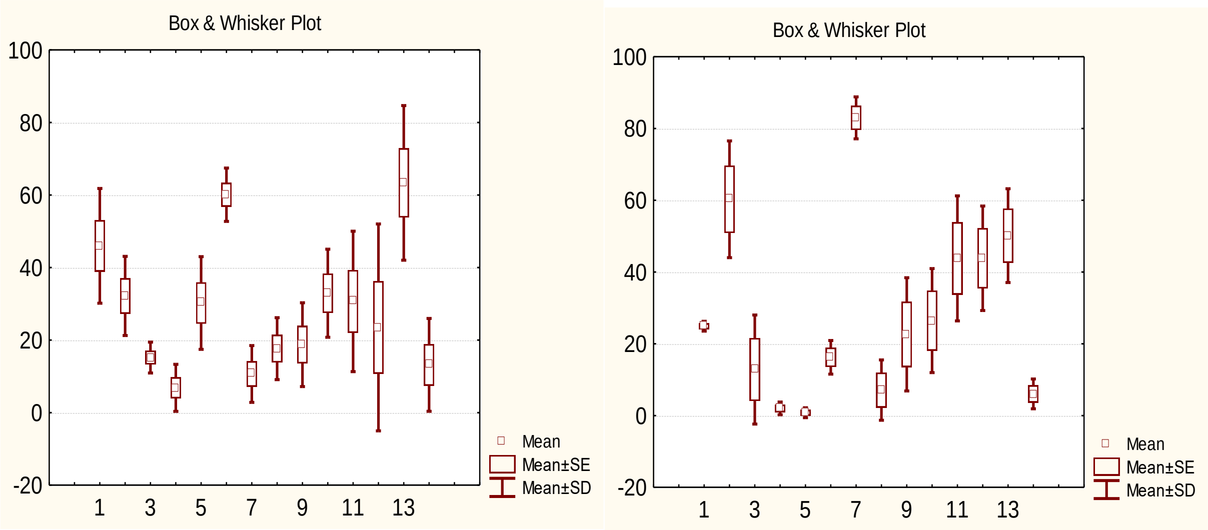
A wide range of intrapopulation recombination variability was revealed in all hybrid combinations for all the traits studied (Fig. 12 *a*, *b*).

In hybrid populations of the 1st group, the frequency of occurrence of genotypes that form side shoots in each node of the plant or every other node, depending on the hybrid combination, is very high and varies from 30.7 to 60.4%, the average for the group beings 46%. There are very few genotypes without side shoots or forming single shoots in the population of each combination – 4.8%, 1.4%, 2.3%, 8.3%, and 7.4%, respectively (Fig. 12 *a*).

The data recorded and analysis of the trait ***length of internodes*** showed that 60% of genotypes in populations had internodes of average length (from 6.1 to 9 cm), which means more inflorescences on the stem. Long internodes (10.6-12.5 cm and 9.8-12.5 cm) formed 36.5% of the splitting population from the combination L11069 x L556 and 47.8% in the combination L11069 x L111, while plants with very short internodes (<6 cm) were observed in small numbers (average 5.8%) in these hybrid populations. A wide range of variability was observed in the flower *morphology* (Fig. 12*a*). The fewest genotypes (11.4 and 8.4%) with various kinds of flower deformations were identified in the populations of hybrids L11069 x L556 and L11069 x L111; normally developed flowers were found in 42.3 and 56.4% of genotypes, respectively. Diametrically opposite data were obtained for the combinations L11069 x L828 and L11069 x L187, in populations of which 65.9% and 41.5% of genotypes were characterized by strong and moderate disturbances in the structure of the flower or its individual elements. Approximately 1/3 of the genotypes have heterostyly and even parthenocarpy. Consequently, fruit set was weak, there were no seeds at all or very few of them (up to 25 pieces), which indicates the presence of pleiotropic effects. Plants with single or complete absence of side shoots with short internodes were also identified (from 22.7 to 50.1%, respectively).

In the populations of hybrids of the first group, the predominant part of the genotypes formed small fruits (from 40 to 70 g). The range of variability in populations from different combinations is quite wide. In the populations from the combinations L11069 x L 28 and L11069 x L111, 69.5% and 32.1% of genotypes had large fruits, respectively.

In the segregating populations F_2 hybrids of the second group, many genotypes are found in the populations that form side shoots every 2-3 nodes (Fig. 12 *b*). No plants without side shoots were found in the population from the combination Mo443 x variety Fakel. A low percentage of genotypes (2.6 and 3.4%) forming 1-2 underdeveloped reduced side shoots was also detected in the populations Mo443 x MaKrista and Mo443 x MilOranj. Most of the genotypes in the segregating hybrid populations of this group had short internodes (Fig. 12 *b*).



a ***b***

Fig. 12. Recombination variability for the traits controlled by mutant marker genes *ls* and *br* in segregating F_2 populations from crossing with line *11069(a)* and mutant form *Mo 443* (*b*)

Note: Traits – 1. High degree of side shoot formation (100-71%). 2. medium (70-41%). 3. low (40-10%). 4. very low (<10%); 5. Long internodes (12.5-9.1 cm). 6. internodes of medium length (9.0-6.1 cm). 7. short internodes (<6 cm); 8. A high degree of morphological disturbances in the flower structure (100-71%). 9. media degree of morphological disorders (70-41%). 10. low level of disturbances in the flower structure (40-10%). 11. normally developed flowers (<10%); 12. Fruit weight, medium to large (120-71 g). 13. From medium to small (70-40 g). 14. small to very small (40-10 g).

Probably, the genetic determination of this type of growth of these forms predetermined the pattern of its manifestation. Significant differences have been found both between hybrid combinations and the range of intrapopulation variability in the degree of disturbances in the flower structure. For example, in the populations F_2 – Mo443 x MaKrista and Mo443 x Fakel, the frequency of occurrence of genotypes with normally developed flowers is high (60.2% and 45.7%), while in the population Mo443 x MilOranj, there are fewer of them (25.5%) and the frequency of occurrence of genotypes with various types of deformations of the flower and its elements, including sterile ones, is 56.7%. For fruit weight, the range of variability is narrowe, genotypes with larger and more uniform fruits predominated in the populations (Fig. 12 *b*).

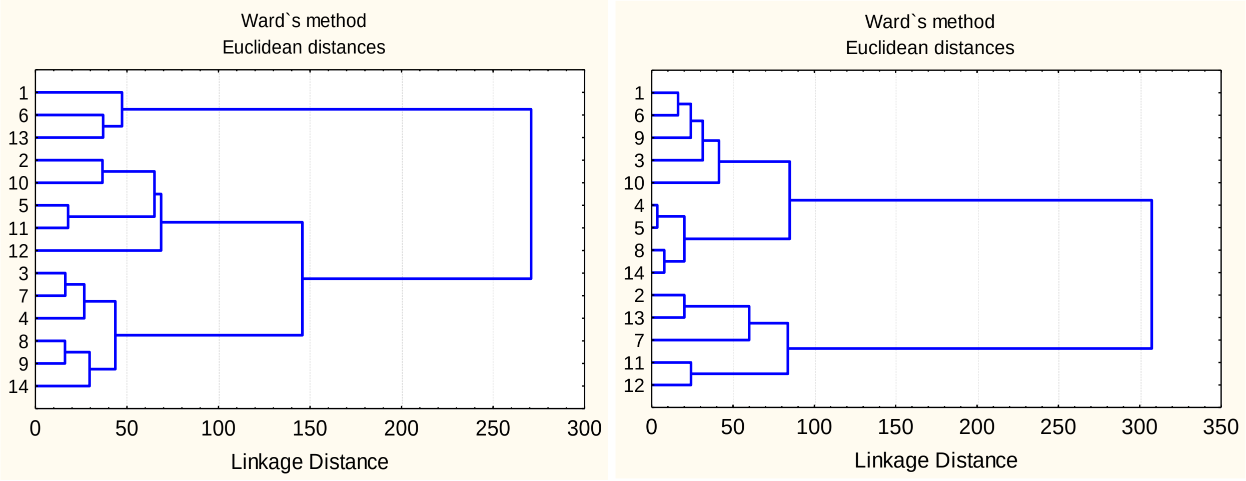
A wide range of intrapopulation recombination variability in the degree of manifestation of the traits controlled by the mutant *ls* and *br* genes in F_2 revealed the need to systematize genotypes with a certain combination of traits in a cluster complex. The genotypes divided into separate clusters are characterized by a very complex combination of traits (Fig. 13 *a, b*), while intrapopulation similarities and differences have been also found. They are quite pronounced both for the traits studied and their combination in one genotype, and the hybrid populations derived from crossing indeterminate (1st group) and determinate forms (2nd group).

For the first group of hybrids, the most numerous first cluster included genotypes with strong shoot forming ability (from 71 to 100%, almost on the node of every leaf of the plant), internodes of medium length (6.1-9.0 cm), small and medium weight fruits (41-70 g) (Fig. 13 *a*). This combination of traits indicates the ambiguous influence of these genes on their expression in these descendant populations. Depending on the shoot forming ability of the parental forms, these traits are determined to a greater or lesser extent by the cultural genome. The second cluster included genotypes with a more complex combination of traits: an average degree of formation of side shoots (41-70%) with long internodes (9.1-12.5 cm), fewer morphological deviations in the structure of flowers (0-40%) and large fruits (up to 120 g). The 3rd cluster contains genotypes with the most complex combination of traits, low side shoot forming ability (one per 4 nodes) or their complete absence on the main stem (0-40%), short internodes (<6 cm), a high degree of disturbances in the flower structure (from 41 to 100%) and small fruits (from 10 to 40 g) (Fig. 13 *a*). From the above, the conclusion is as follows – *the fewer side shoots are formed and the shorter the internodes on the plant, the more morphological deviations in the structure of the flowers and the smaller the fruits. This indicates a multiple (pleiotropic) effect from the influence of the* ***ls*** *and* ***br*** *genes due to their insufficient cultivation, or these side effects are due to the linkage of these genes with others, which have an indirect effect, enhancing the pattern of the manifestation of mutant traits*. In tomato, many mutant genes that control significant breeding valuable traits act pleiotropically [21, 32]. Suppression of their negative effects is possible with more active cultivation of the mutant forms of which they are carriers.

Clustering of genotypes of the 2nd group of hybrids obtained with the mutant form Mo 443 (*ls*) revealed similar, different degrees of similarity and differences between genotypes in splitting hybrid populations, according to a certain combination of traits, including depending on the traits of the parental forms used in crosses.

According to the degree of manifestation of traits and their specific combination in one genotype, they are also distributed into three clusters (Fig. 13 *b*).

The genotypes of the third cluster with an average degree of formation of side shoots (41 - 70%), with short internodes (<6 cm), normally developed flowers and medium to large fruit weight (from 70 to 140 g) present the highest interest.



a ***b***

Fig. 13. Dendrograms of similarities and differences of genotypes with a certain combination of traits controlled by mutant marker genes isolated from segregating populations of different hybrid combinations with *L 11069 (a)* and mutant form *Mo 443 (b)*

Note: Traits – 1. High degree of side shoot formation (100-71%). 2. medium (70-41%). 3. low (40-10%). 4. very low (<10%); 5. Long internodes (12.5-9.1 cm). 6. internodes of medium length (9.0-6.1 cm); 7. short internodes (<6 cm); 8. High degree of morphological disturbances in the flower structure (100-71%). 9. media degree of morphological disorders (70-41%). 10. low level of disturbances in the flower structure (40-10%). 11. normally developed flowers (<10%); 12. Fruit weight, medium to large (120-71 g). 13. medium to small (70-40 g). 14, small to very small (40-10 g).

The high recombination variability in the pattern of manifestation and degree of expression of the traits controlled by the *ls (lateral suppresser)* and *br (brachytic*) genes in segregating F_2 populations, and the clustering of genotypes with a certain set of traits showed that the two genes *ls* and *br* have a stronger negative effect on manifestation of the traits of the reproductive system than one *ls* gene, which indicates their complementary action.

Analysis of variance of genotypic variability in F_2 offspring from different combinations (8) for the parameters of the traits (4) with varying degrees of manifestation (14 indices), controlled by the *ls* and *br* mutant genes, showed an excess of intercluster variance over intracluster one (Table 19). This indicates significant differences between the analyzed hybrid combinations and high intrapopulation recombination variability within each of them in the pattern of manifestation and degree of expression of the traits, as well as the successful differentiation and systematization of genotypes, for a set of traits with a certain value of indices in clusters (Table 19). Their systematization based on breeding value will allow optimizing programs when developing new initial material.

Table 19. Analysis of variance of genotypic variability in segregating F_2 populations for the traits controlled by the *ls* and *br* genes

F_2 hybrid combinations	Intercluster variance	df	Intracluster variance	df	F	P
	indeterminate × indeterminate combinations					
11069 x 187	4763.371	2	1419.867	11	18.45141	0.000306
11069 x 828	4284.437	2	2577.780	11	9.14136	0.004585
11069 x 556	8005.041	2	10,53.736	11	41.78250	0.000007
11069 x 28	2616.694	2	1733.123	11	8.30398	0.006338
11069 x 111	3347.385	2	1649.203	11	11.16334	0.002251
	determinate × superdeterminate combinations					
Mo 443 x c. MaKrista	6024.723	2	1553.766	11	21.32623	0.000164
Mo 443 x c. MilOranj	5093.761	2	2428.767	11	11.53494	0.001994
Mo 443 x c. Fakel	8916.381	2	2945.368	11	16.64991	0.000470

6.4. Multi-marker tomato lines and the degree of trait expression controlled by the *ls* and *br* genes in F_3- F_5 populations

In the third generation, the offspring of genotypes isolated in F_2 from different combinations of hybrids of the first group showed cleavage in all traits. This is most pronounced in the formation of side shoots and morphological deviations in the structure of flowers in the F_3 offspring from the combination L11069 × L556 (the split was similar to the F_2 population), which indicates the instability of the genetic systems of the original forms, as well as side effects from the *ls* and *br* genes due to *linkage* with other genes that enhance the manifestation of mutant traits. In the F_3 populations from the combinations L11069 x L187 and L11069 x L828, there were more semi-cultivated genotypes with small fruits (32-45 g), single side shoots and pronounced deviations in the structure of the flower. At the same time, genotypes with traits of cultivated lines (more medium-sized side shoots, long internodes, larger fruits) emerged, as well. Aligned offspring for selectable traits in F_3 were obtained from genotypes isolated from the populations L11069 x L28 and L11069 x L111. Lines 28 and 111 have a semi-determinant growth type ($sp^{\pm}$) and are carriers of the *rin* and *nor* storability genes. From the F_3 populations of these combinations, more genotypes (lines) were isolated that successfully combine limited formation of side shoots, short and medium-length internodes, normally developed flowers, medium-sized and large fruits, and stable manifestation of these traits in F_4.

Populations more aligned in terms of the degree of manifestation of traits in F_3 offspring form genotypes isolated from hybrid combinations of the 2nd group. A stable pattern of manifestation of selected traits was observed in populations from Mo 443 × MaKrista and Mo 443 × Fakel. Strong segregation for reproductive system traits was found in populations of F_3 offspring from genotypes derived from the combination Mo 443 × MilOranj.

Multi-marker lines 196, 197, 204, 224, and 228 (Table 20), produced in F_4 from the combinations L11069 x L28 and L11069 x L111, are valuable in breeding terms. The plants are of a semi-determinant growth type, mid-ripening (101-110 days), with a main stem length of 110-138 cm, forming weak, self-limiting growth side shoots. The number of side shoots on the main stem ranges from 3.0 to 6.4 per 18.2-21.7 nodes, internodes of average length, from 6.7 to 9.6 cm (Table 20). These lines in F_4 and F_5 in two years different in climatic conditions (2015, 2016) showed a stable pattern of manifestation of the analyzed traits, although plants with small fruits were produced, but without side shoots in some offspring. The plant habitus is characterized by an erect to semi-erect stem with a good leaf to fruit ratio. The color of the leaves is dark green, of different shapes, ovate, elongated-ovate, broadly lanceolate. Differences were also revealed in the shape and morphology of the flower, normally developed and flowers with a full corolla, but petals fused into a tube.

Another group of lines (186, 205, 237, 241, 10/16) are the offspring of the genotypes isolated from the hybrid combinations L11069 x L 187, L11069 x L828 and L11069 x L556. They have an indeterminate growth type with an optimal combination of breeding traits (Table 20). For example, line 186 forms a limited number of side shoots on the plant (3.3-3.8 per 28.8-32.9 nodes), has large and dense fruits (110 g or more). Line 237 is distinguished by the identical traits. Weak indeterminate growth, close to semi-determinate, but not self-limiting with a frequent arrangement of inflorescences, are characteristic of lines 205, 241 and 10/6. They have 3.8 - 6.1 side shoots per 21.4-29.4 nodes (levels) and on some nodes, there are from 3 to 7 very small, reduced side shoots (3-4 cm). Lines (110, 164, 178, 181, 183, 188, 193, 195) of super determinate and determinate growth types, with aligned inflorescences, normally developed flowers, low side shoot forming ability, dense and uniform by shape and color of fruits were isolated from combinations with the mutant form Mo 443 (*ls*), (Table 20). This indicates that the initial mutant form (Mo 443) had a high polygenic content for these traits, since the ls gene does not have a strong negative effect on their manifestation. Analysis of the populations of offspring of the F_4 and F_5 generations shows that the pattern of the manifestation of traits was stable in 2016 compared to 2015, (Table 20).

Initial material (multi-marker lines) with different combinations of genes (*sp*$^+$, *sp*$^\pm$, *sp, ssp, rin, nor, ls, br, pis, ex, sl* etc.) was developed, that form a limited number of side shoots with short internodes, different in the type of growth and habitus of plants, the duration of the growing season, with different types of inflorescences and sterility, ensuring the possibility of their reproduction, as well as for size, shape, color and quality of the fruits. These lines corroborate with the research objectives and are included in work programs to study their combining ability and develop F_1 tomato hybrids.

6.5. Variability of pollen quality and quantity in multi-marker tomato lines depending on the level of inflorescence laying and high temperature

Marker traits *(ssp, sp, sp*$^+$*, sp*$^\pm$*, br, ls, pis, nor, rin, u, gs. j, j-2)*, the carriers of which are the lines developed, are easily identified in open and protected ground (greenhouse) at different stages of plant growth and development. A limitation to their active inclusion in breeding programs may be the quality of their pollen, since they are obtained from crossing forms of carriers of the *ls* gene, which is associated with morphological deviations in the structure of the flower. Therefore, we studied the fertility and viability of pollen, the morphological traits of pollen grains, pollen productivity, the influence of the

Table 20. Indices of the traits controlled by the *ls*, *br* genes in new multi-marker tomato lines and the stability of their manifestation in the F_4 (2015) and F_5 (2016) generations

No lines (genotype)	Average number of internodes per plant, pcs		Average length of internodes, cm		Number of side shoots on the plant, pcs		Number of inflorescences on a plant, pcs		Productivity, kg/plot		Fruit weight, g	
	2016	**%, by 2015**	**2016**	**%, by 2015**	**2016**	**%, by 2015**	**2016**	**%, by 2015**	**2016**	**%, by 2015**	**2015**	**2016**
L 110 *(ssp, u, j, ls, nor, br)*	7.0	100.1	6.8	97.1	4.1	136.2	4.3	97.7	9.4	90.3	91.7	106.4
L 183 *(ssp, u, nor, ls)*	6.8	97.0	6.4	91.4	3.0	115.3	3.7	115.5	10.2	102.5	81.0	70.5
L 188 *(ssp, u, t, ls)*	9.0	100	7.0	97.2	3.3	97.3	4.1	124.5	9.7	104.3	68.0	80.7
L 164 *(sp, u, ls, j-2, rin)*	10.1	98.0	8.5	98.8	3.1	103.0	5.4	101.9	11.6	97.7	68.6	75.8
L 178 *(sp, u, ls)*	9.0	96.6	9.0	91.6	4.2	89.4	4.7	104.4	13.8	100.3	94.7	101.0
L 181 (*sp, hp-2, j-2, u*)	10.3	97.0	8.1	87.7	3.2	91.4	4.5	102.4	12.6	97.9	78.0	88.6
L 193 *(sp, ls, u, nor)*	9.6	91.4	8.5	88.2	3.1	103.3	5.1	124.3	11.9	92.2	64.3	73.2
L 195 *(sp, u, ls, nor)*	10.1	96.2	7.0	97.1	3.5	95.8	4.2	105.0	13.5	101.5	65.0	76.0
L 196 (*sp*$^{\pm}$, *ps, r, c, ls, br*)	21.2	100.7	9.6	110.2	3.0	115.3	4.6	109.5	12.3	99.7	67.0	78.4
L 197 (*sp*$^{\pm}$, *ls, t, rin*)	21.3	87.3	8.6	89.7	5.1	83.7	6.1	96.8	14.6	95.6	85	92
L 228 (*sp*$^{\pm}$, *ls, u, ex*)	21.7	88.2	9.0	100	6.4	78.3	7.4	98.2	12.9	96.6	63.7	76.2
L 234 (*sp*$^{\pm}$, *u, nor, ls, br*)	21	115.3	6.7	94.8	3.4	77.3	7.1	110.9	14.7	113.7	74.6	86.8
L 186 (*sp*$^{+}$, *ls, br, u, nor*)	30.1	101.0	8.3	100	3.8	106.6	11.5	121.3	21.7	103.3	111.3	119.3
L 205 (*sp*$^{+}$, *ls, u*)	29.4	104.6	9.0	86.3	5.3	106.0	9.8	101.2	18.6	99.8	43.8	61.5
L 237 (*sp*$^{+}$, *rin, ls, u*)	28.2	102.4	10.5	98.0	4.4	104.7	9.4	100.8	19.1	95.6	72.8	89.2
L 241 (*sp*$^{+}$, *ls, u, rin*)	28.4	88.0	8.8	105.6	6.1	101.8	8.9	96.7	15.9	103.5	112	118.3
L 10|16 (*sp*$^{+}$, *ls, gs, rin*)	21.4	87.3	8.4	96.7	5.2	92.8	7.0	94.6	11.0	96.5	20	14.5

level of inflorescence set on the plant and high temperature on the variability of these traits, since reproductive functions depend on them.

The study and analysis of pollen populations of multi-marker lines based on the ratio of sterile and fertile pollen grains in them showed high fertility in almost all lines (from 70.4 to 96.1%). Assessment of pollen viability, which characterizes the fertilizing ability of a pollen grain, did not reveal a direct relationship between fertility and viability. For example, L 234 with pollen fertility of 93.6% has a viability of 16.7%, while L 204 with fertility (78.5%) has a pollen viability of 45.9%. Lines 196 and 228 with the lowest pollen fertility had viability of 19.9% and 30.0%, respectively. Hence, there are significantly fewer pollen grains capable of germinating and producing fertilization than fertile ones in the pollen populations of the studied lines. Simultaneously, pollen from multi-marker tomato lines was assessed for resistance to high temperature and drought. Genotype specific differences between the lines were recorded in the response of their pollen to the action of high temperature and osmotic factor (simulating drought). Regarding the response of pollen lines to the action of high temperature factors, the indices within the group varied significantly from 18.0% in L 234 to 83.1% in L 193. There were also pronounced differences in pollen germination of the studied lines against the background of the osmotic factor, from 0.1% in L 183 to 55.9 in L 110. Analysis of variance of the sources of variability in pollen resistance to the stress factors studied revealed the share of the influence of genotype, stress factors and their interaction in the total variability of traits (Fig. 14).

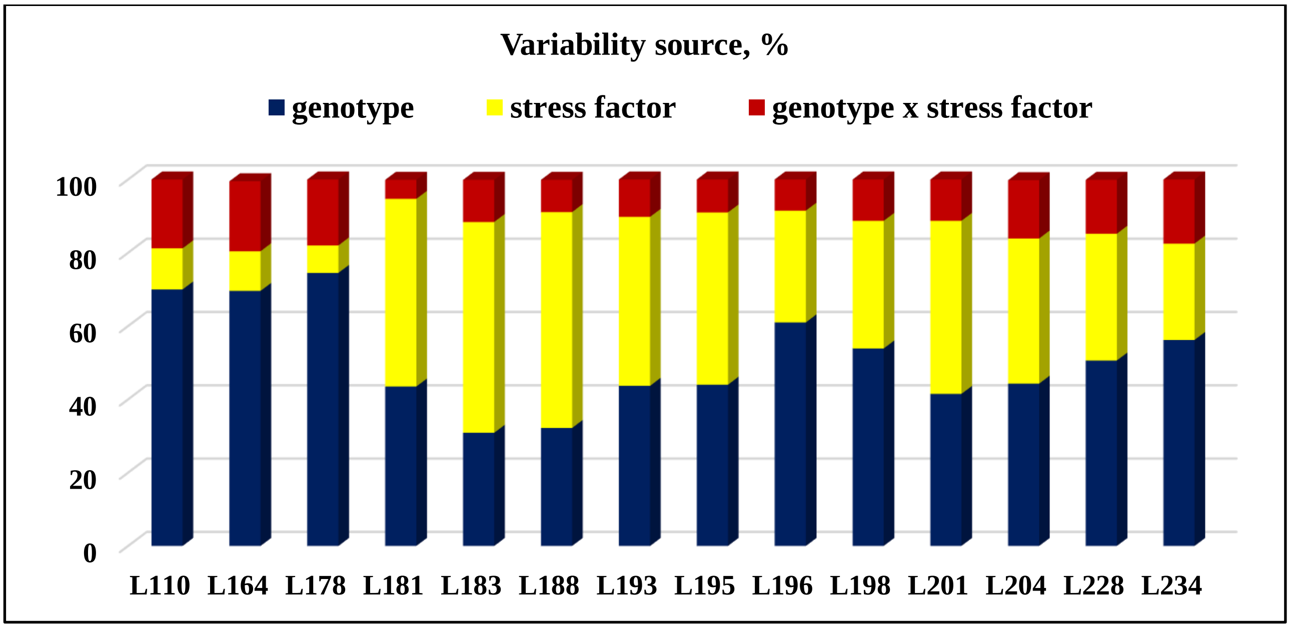


Fig. 14. Factor analysis of the variability sources of pollen resistance to abiotic stress in multi-marker lines

The ***influence of the level of inflorescence set on the main stem of the plant on pollen viability and its resistance to high temperature*** was studied in addition to these characteristics. Differences in the viability of freshly collected pollen (control) were observed both between lines and depending on the level of inflorescence initiation. Pollen collected from flowers of inflorescences of the lower tier (1-2 racemes) had high viability (55.2% - 40.6%), on the 3rd-4th inflorescences this index is even higher (64.3% - 58.8 %), two or more times lower (14.5% - 26.0%) on the 5th and 6th inflorescences. In all studied lines, high viability was characterized by pollen obtained from flowers of inflorescences of the middle tier (3-4), and the lowest was observed in all lines on the inflorescences of the upper tier of the plant (Fig. 15 *a*). The same regularity was found for the trait “pollen tube length” (Fig. 15 *b*). It should be noted that the inflorescences of the middle tier (3-4) form and bloom under the most favorable environmental conditions, while the inflorescences of the upper tiers (5-6) are subject to critical conditions in terms of temperature and moisture deficiency.

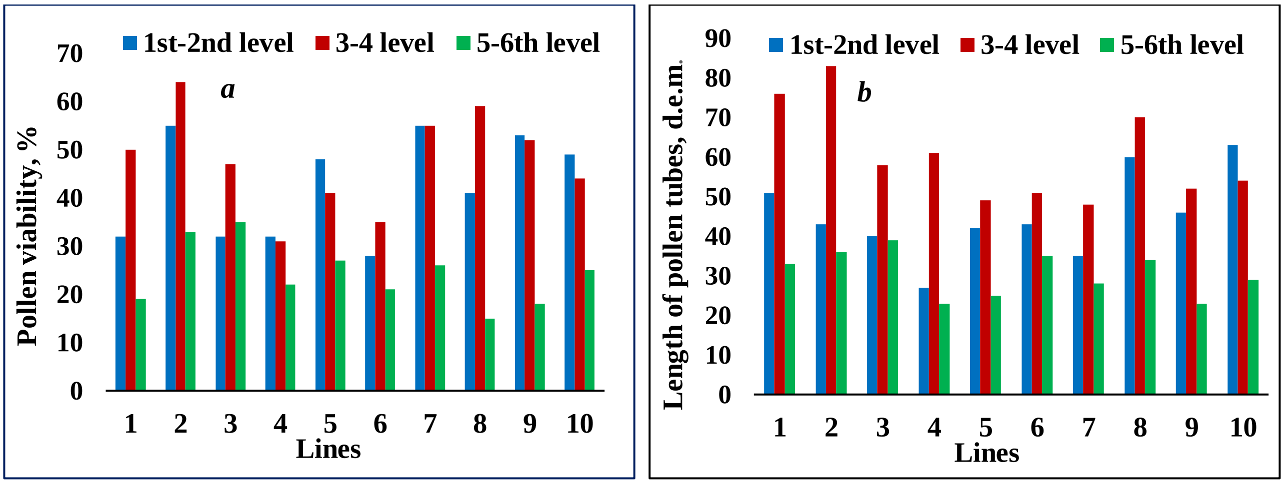


Fig. 15. Indices of pollen traits in lines depending on the level of location of the inflorescence on the main stem of the vegetative plant (control): ***a – pollen viability, %; b – length of pollen tubes, d.e.m.***

The regularities were slightly different when pollen of lines obtained from flowers of inflorescences of different tiers was treated at a temperature of 45˚C for 8 hours, followed by its germination on an artificial nutrient medium under *in vitro* conditions. A high differentiated response of pollen lines was revealed, both in general and to high temperature exposure and, in particular, depending on the level of inflorescence position on the plant (Fig. 16 *a, b*). Analysis of the trait “stability of pollen tubes” shows that pollen from flowers of inflorescences of the upper tier (5-6) germinates faster after heat treatment (45˚C) (Fig. 16 *a*), simultaneously forming very long tubes (Fig. 16 *b*), while in the control they were the shortest (Fig. 15 *b*). This indicates that pollen from the flowers of the upper tier of inflorescences, which was formed at high temperatures, in the populations studied has a larger number of resistant gametes, which, when germinating, simultaneously form long pollen tubes that can ensure fertilization under conditions of high temperature stress.

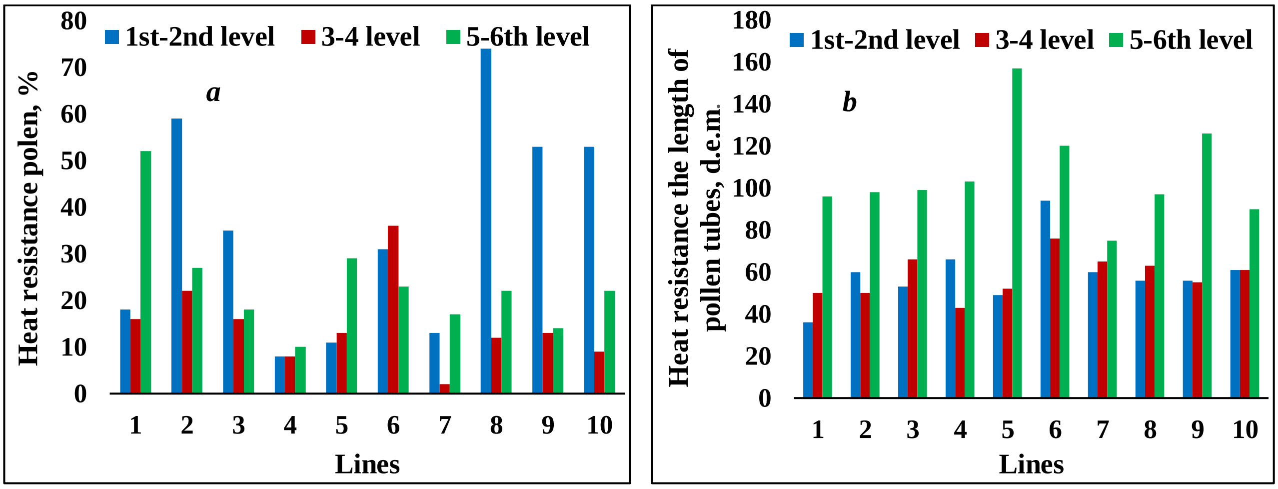


Fig. 16. Variability of pollen trait indices in tomato lines depending on the level of inflorescence position and high temperature: ***a – heat resistance for pollen germination, %; b – length of pollen tubes, d.e.m.***

The study and analysis of the ***morphobiological characteristics of pollen grains in tomato lines*** before and after heat treatment, depending on the level of inflorescence initiation, revealed particular features characteristic of each line, both depending on the location of the inflorescence and the response to temperature stress (Table 21). A specific pattern in the variability of the trait “pollen grain size” depending on the level of inflorescence position on the plant and the response of pollen lines to high temperature has not been established. The differences are determined by the genotypic particular features of the lines. Some (1, 2, 10) form large grains on all inflorescences of the plant, but have a strong response to high temperature, decreasing in size. Others (6, 7), on the contrary, having very small pollen, do not react at all to high temperature. At the same time, lines (5 and 8) were also identified, the pollen grains of which decrease in size as the level of inflorescence on the plant increases; this pattern persists even after high-temperature exposure (Table 21).

Table 21. Pollen grain measurements in tomato lines before and after heat treatment relative to the tier of inflorescence location on the main stem

Lines	Diameter of pollen grains of freshly collected pollen (μm)			Diameter of pollen grains after heat treatment (μm)		
	Inflorescences			Inflorescences		
	1-2	3-4	5-6	1-2	3-4	5-6
1. L 166	28,9±0,11	30,0±0,14	30,0±0,12	22,0±0,20***	29,0±0,16*	27,0±0,11***
2. L 167	30,0±0,18	32,9 ±0,16	28,0±0,14	30,0±0,19	27,6±0,19***	27,6±0,17
3. L 168	26,0±0,20	27,0 ±0,16	27,4±0,18	26,0±0,11	25,0±0,18***	27,0 ±0,14
4. L 169	25,0±0,16	26,3 ±0,21	22,0±0,20	23,0±0,17**	23,0±0,15***	22,0 ±0,15
5. L 170	29,0±0,15	28,0 ±0,11	27,0±0,17	25,0±0,14***	24,0±0,19***	22,0 ±0,12***
6. L 171	20,0±0,10	21,0 ±0,13	20,0±0,10	21,0±0,10*	20,0±0,13*	21,1 ±0,17**
7. L 172	23,0±0,15	23,0 ±0,14	24,0±0,13	23,2±0,18	22,6±0,21	24,0 ±0,13
8. L 173	33,1±0,21	26,0 ±0,15	23,0±0,11	29,0±0,18***	25,6±0,19*	22,4 ± ,12*
9. L 174	26,1±0,17	21,0 ±0,11	25,0±0,15	26,1±0,15	20,8±0,23	24,9±0,11
10. L175	28,0±0,10	29,0 ±0,18	29,0±0,21	19,6±0,10***	20,0±0,13***	23,0±0,09***
The differences are significant: * at the 0.05% level, ** 0.01% and * 0.001%**						

For the trait ***pollen productivity,*** which is of particular importance for tomato lines used in heterotic breeding as pollinators, significant differences were recorded both between the studied lines and within them in the ability to produce a certain volume of pollen per flower, depending on the level of inflorescence formation on plant. Lines 7 (172) and 10 (175) are characterized by high pollen productivity, and these two lines have different characteristics in the size of pollen grains (7-small, 10-large). Low values were observed for lines 1 (166), 3 (168) and 6 (171) (Table 22). It has been assessed that the amount of pollen produced in flowers decreases from the first inflorescence to the last. More pollen is produced in the flowers of the inflorescences of the middle tier (3-4), which is highly viable and, when germinating, forms the longest tubes (Fig. 15 *a, b*).

The differences between the lines in pollen productivity and the percentage of working (functional) pollen, estimated by the amount of pollen produced and its viability, are also quite pronounced. They are also significant relative to the location of the inflorescence on the plant and are most pronounced between the middle (3-4) and upper tiers (5-6) (Table 22).

Table 22. Pollen productivity and working pollen volume in tomato lines depending on the location of the inflorescence on the plant

Lines	**Pollen productivity, mg/flower**				**Working (functional) pollen volume, %**			
	Level of inflorescence location on the vegetating plant							
	1-2	**3-4**	**5-6**	**Mean**	**1-2**	**3-4**	**5-6**	**Mean**
1. L 166	0,311	0,314	0,395	0,340	9,8	15,7	7,3	10,9
2. L 167	0,564	0,571	0,509	0,548	31,1	36,6	16,5	28,1
3. L168	0,396	0,434	0,318	0,382	12,7	22,6	11,6	15,6
4. L 169	0,660	0,702	0,612	0,658	21,2	20,2	13,3	18,2
5. L 170	0,531	0,546	0,404	0,494	25,3	18,2	11,0	18,2
6. L 171	0,276	0,309	0,268	0,284	7,7	10,8	5,7	8,1
7. L 172	1,081	1,280	1,020	1,127	59,2	70,8	26,5	52,2
8. L 173	0,840	0,760	0,893	0,831	34,1	44,7	12,9	30,6
9. L 174	0,712	0,686	0,511	0,636	37,5	35,4	9,2	27,4
10. L 175	1,280	1,420	1,296	1,332	62,5	61,9	32,4	52,3
Mean	0,665	0,720	0,623	0, 667				
NSR_{05}	**0, 043**			**0,094**				

A comprehensive analysis of multi-marker tomato lines revealed the influence of the level of inflorescence initiation on the plant and the high-temperature factor on the nature of variability of male gametophyte traits, pollen viability, pollen tube length, pollen germination resistance, pollen tube length resistance, pollen grain sizes, pollen productivity and percentage of working (functional) pollen. It has been established that each line is characterized by genetically determined individual variability in the indices of the studied traits, the magnitude of which simultaneously depends on the age characteristics of the plant and environmental factors, while different traits of the same genotype vary unequally, having certain limits. All traits of the male gametophyte and each one separately, when analyzed, reveal a large component of variability depending on environmental factors, which is especially important to know when using these lines in heterotic breeding as a paternal component.

GENERAL CONCLUSION AND PRACTICAL RECOMMENDATIONS

General conclusion

1. Artificial stress backgrounds (high and low temperatures, drought) have been developed and optimized to assess the resistance of collection and breeding tomato samples based on the traits of the male gametophyte (pollen) under *in vitro* conditions; a resistance scale for differentiation, systematization of screening results, and a scheme for organizing studies on the genetic potential of the mutant gene pool have been worked out (Chapter 2, [146]).

2. A method has been developed for assessing the adaptive potential of tomato genotypes to high temperature stress using severe conditions: 38˚C; 45˚C; 48˚C and temporal exposures of plant pollen of different durations (3, 5, and 7 hours), which can be widely and efficiently employed in breeding to select resistant forms at the initial stages (Chapter 3, [130]).

3. It has been found that pollen of tomato genotypes is able to endure low temperatures (+6˚C) for a long time (60 days) maintaining a relatively high percentage of viable (10.0-12.0%), functional (tube length of 28-36 d.e.m.) pollen, which proves the efficiency of the method for selecting microgametophyte resistant forms and its employment in seed production of heterotic hybrids when it is necessary to store pollen for multiple pollinations (Chapter 3, [339]).

4. Screening of resistance of single marker and multi-marker mutant forms of tomato (125 samples) to three abiotic stress factors, high and low temperatures and drought using a set of traits of the male gametophyte has demonstrated that resistance to them is determined by different genetic systems, revealing differences even within one mutant form for pollen germination and the formation of a pollen tube of sufficient length for fertilization (Chapter 4, [134]).

5. The potential of the gene pool of tomato mutant forms is shown for breeding valuable traits, at the stage of seedlings, cotyledons and first true leaves, controlled by a large number of genes as follows: *a, aa, aw, ag, al, hl,* and *ls* ***at the seedling stage*** and *aut, apn, alb; gil, Cu, cg, inta, Ln, lu, ltf, lg-2, Me, marm, nv, pu2, pl, res, sf, sy, V-5/+,* and W^{om} at ***the stage of cotyledon and first leaves****;* ***for plant growth and habitus –*** sp^{+}*;* $sp^{\pm}$*; sp; ssp; d; dd; dmd, wd, ls, atn, bu, br, cg,* and *bip,* the length of the growing season from ultra-early ripening (83-99 days) to ultra-late ripening (131-158 days); *ex, ps, ps-2, pi, pi-2, sl, sl2, ch, glf, cs-2,* and *Ge* ***for sterility****; rin, nor, alc, u, gs, gf, f, bk, ck, anr, ptb, at, β, Lc, Ip, Del, Abg, hp-1, lo,* and *loc* are genes that control the ***density, color, shape, size, chambering of fruits***, etc., which determines a wide range of vital forms and is of great interest and value as a new germplasm for solving problems of practical breeding and conducting fundamental research (Chapter 4, [135]).

6. It has been found that the manifestation of morphological (diameter, perimeter, and area) and cytochemical characteristics (DNA amount, chromatin density) of the nuclei of vegetative (V) and generative (G) pollen cells in F_1 hybrids depends both on the characteristics of the parental forms and the effect of temperature stress factors on pollen (45 ºC and +7 ºC), which in almost all F_1 tomato hybrids

studied is intermediate, or the value is close to the less resistant parental form, which indicates the recessive nature of their inheritance (Chapter 5, [123, 126]).

7. The regularities of inheritance (hp) of resistance to high and low temperatures by F_1 tomato hybrids for the traits of the male gametophyte have been established. The analysis of their heritability (h^2) has shown that the resistance of F_1 hybrids is determined by the maternal forms; at a higher level and prolonged exposure to the stress factor on pollen, the influence of the interaction of the parental components increases, while the contribution of the paternal forms is insignificant. This can serve as a basis for a reasonable selection of parental pairs in the development of highly resistant varieties and heterotic hybrids (Chapter 5, [127, 132]).

8. A method has been developed for selecting resistant transgressive recombinants from splitting tomato F_2 populations for a set of male gametophyte traits against the background of high temperature (45ºC) based on the particular features of the combination of traits expressed in the average variance index for the hybrid family (S^2), which makes it possible to isolate resistant recombinants and carry out selections at the early stages of breeding taking into account other economically valuable traits, as well (Chapter 5, [128, 149]).

9. The efficiency of the combined employment of gamete and traditional breeding methods based on the step-by-step alternation of selections for sporophyte and gametophyte against the background of high temperature stress (43˚C and 45˚C) at the early stages in F_1, F_2, F_3 resulting in intensification of the breeding process and producing offspring (lines) in F_4 - F_6, which are more resistant, productive, with a short growing season, low initiation of the first inflorescence, lower flower shedding and high fruit set under high temperatures has been substantiated on a scientific basis (Chapter 5, [146, 148]).

10. High recombination variability has been revealed in the pattern of manifestation and degree of phenotypic expression of the traits controlled by the mutant genes *ls* (*lateral suppresser*) and *br* (*brachytic*) in segregating F_2 populations, cluster analysis showing that their combined effect (*ls* and *br*) has a stronger negative impact on the reproductive trait manifestation than single *ls* gene, which indicates their complementary action (Chapter 6, [135]).

11. It has been established that the lower number of side shoots and the shorter the internodes on a tomato plant, the more morphological deviations in the flower structure and the smaller the fruits, which indicates the multiple pleiotropic effect of the *ls* and *br* genes due to their insufficient culture level. Negative effects can also be determined by their *linkage* with other genes that enhance the degree of mutant traits manifestation (Chapter 6, [135]).

12. Multi-marker lines (>50) with an original combination of traits controlled by the sp^{+}, $sp^{\pm}$, *sp, ssp, c, rin, nor, ls, br, pis, ex, sl, gs, hp-1, j- 2, j* genes have been developed. These genes are expressed at different ontogenetic stages and characterize the type of growth and architectonics of the plant, internode length, shoot forming ability (without runners, with few runners), reproductive system (type of sterility), fruit traits (size, shape, color, marketability) and overall productivity. It has been demonstrated that the quality of pollen, its productivity and the percentage of functional pollen in these lines depend on the layer of inflorescence set on the plant

and the action of the high temperature factor, but the degree of variability of trait indices is determined by the genotype (Chapter 6, [135, 150]).

13. Differentiating capacity of the developed methodological approaches regarding the assessment of resistance to abiotic stress factors based on the traits of the male gametophyte, the study of variability and inheritance of traits together with different combinations of cultivated and mutant forms of tomato, analysis of their offspring (F_1-F_{10}) at different stages of ontogenesis (sporophyte, gametophyte) has made it possible to produce more than 100 lines incluing gene carriers of the traits valuable for breeding and new varieties, *MaKrista, MilOranj, Stefani, Prichindel, Vivat, Dimetra, Ilica, Cireasca, Matriona, Petramak* (Chapter 5 [151, 152, 153, 154, 155 , 156, 157, 158, 159, 160]), as well as heterotic hybrids, two of which, *Ingstar* and *Rozamak*, are being tested by the State Variety Testing Commission of the Republic of Moldova (Chapter 6, [161, 162]).

Practical recommendations

1. It is recommended to involve tomato mutant forms in the breeding process as new germplasm, which will contribute to expanding the range of genotypic variability in populations, thereby making it possible to accomplish many objectives in view of producing plant forms with new architectonics (differing in determinate type, single-stem, with shortened internodes, etc.) and reproductive organs of different structure, with different color, shape, fruit weight and long-term preservation of commercial qualities.

2. To intensify the breeding process in the development of early-ripening and later-ripening varieties capable of realizing the genetically inherent productivity potential under high temperature conditions, it is recommended to use combined methods with step-by-step alternation of sporophytic and gametophytic selections with simultaneous assessment of resistance and economically valuable traits.

3. When selecting parental pairs for crossing to create heterotic tomato hybrids resistant to high and low temperatures, it is recommended to use stress-resistant genotypes as the maternal component, hybrids involving such forms have a higher adaptive potential.

4. In crossings and, especially, in reproduction of heterotic hybrids and in industrial hybrid seed production, it is recommended to take into account the location of the flower on the inflorescence and the inflorescence on the main stem of the plant in the paternal component, since age-related changes in combination with the particular features of weather conditions are reflected in the pattern of the trait manifestation in male gametophyte (pollen).

5. Multi-marker lines with different types of sterility ensuring the possibility of their reproduction, different in the type of growth and duration of the growing season with limited shoot formation, short internodes, differing in size, shape, color, marketability of fruits and a number of other economically valuable traits including yielding capacity are recommended to be employed as sources of breeding valuable traits in heterotic breeding.

6. Recommended for cultivation:

(a) salad varieties of indeterminate and determinate growth types, *Stefani, MaKrista, MilOranj, Matriona, Petramak, Vivat, Dimetra, Ilica* (large fruited with pink, orange and red fruits) for open soil production conditions and unheated film greenhouses,

(b) varieties of ornamental type, *Prichindel* and *Cireaşca* (small fruits for fresh consumption, dish decoration and whole fruit canning) for loggias, balconies and open soil according to a compacted scheme;

(c) high-tech indeterminate, mid-early and mid-ripening heterotic tomato F_1 hybrids, *Ingstar* and *Rozamak.* for large scale greenhouses.

BIBLIOGRAPHY

1. АВДЕЕВ, А.Ю. Спонтанные мутации томата по размеру, форме, массе плода и эволюции признака. В: *Доклады РАСХН.* 2014. № 1. с. 16-19. ISSN: 0869 – 6128.
2. АЛИЕВ, Р. Т., ГАДЖИЕВА, П. И., ДЖАВАДОВА, Л. Г. и др. Изменение структурного состояния ДНК в листьях пшеницы в связи с засухой В: *3-й Съезд Всероссийского общества физиологов растений.* С.-Петербург, 24-29 июня, 1993. Вып. 1. с. 5.
3. БЕКОВ, Р.Х. *Создание исходного материала томата с использованием генетических маркеров и эффективные пути его применения в селекции:* Дисс. докт с.-х. наук. Москва. 2012. 461 с.
4. БОЙКО, Е. В., БАДАЕВ, И. С., МАКСИМОВ, Н. Т., ЗЕЛЕНИН, А. В. Изменение количества ДНК при аллополиплоидизации (Закономерности становления и организации генома злаков). Сообщение 1. В: *Генетика.* 1988. 24, № 1. с. 89-97. ISSN: 0016 – 6758.
5. БОЧАРНИКОВА, Н.И. *Генетическая коллекция мутантных форм томата и её использование в селекционно-генетических исследованиях.* Москва. 2011. 120 с. ISBN: 978-5-901695-46-3.
6. ВОЛКОВ, Р.А, КОСТЫШИН, С.С, МИРОШНИЧЕНКО, Г.П. Изменения повторяющихся последовательностей ДНК растений при межвидовой гибридизации. In: *Общая биология.* 1989. Т. 50, № 4. с. 446-457. ISSN: 0044 – 4596.
7. ГАНЕВА, Д., ДАНАИЛОВ, Ж. Успехи болгарской гетерозисной селекции томата. В: *Селекция семеноводство овощных культур.* Москва. 2015. с. 201-208. ISBN: 978-5-901695-65-4.
8. ГОЛУБИНСКИЙ, И.Н. *Биология прорастания пыльцы.* Киев: Наукова думка, 1974. 368 с.
9. ГУСЕВА, Л.И. *Методы селекции томата для интенсивных технологий.* Кишинев. 1989. 223 с. ISBN: 5-376-00180-6.
10. ДАНАИЛОВ, Ж. *Селекция и семеноводство на домати – история, методы, постижения и тенденции.* Изд-во Марин Дринов при Болгарской АН, 2012. 265 с.
11. ДОСПЕХОВ, Б.А. *Методика опытного дела.* Москва. 1985. 416 с. ISBN: 978-5-458-23540-2.
12. ДРАГАВЦЕВ, В.А Новые подходы к экспрессной оценке генетической (аддитивной) дисперсий свойств продуктивности растений. В: *Вавиловский журнал генетики и селекции.* 2012. Т. 16. № 2. с. 427-436. ISSN: 2079 – 0597.
13. ЕРМАКОВ, И.П., МОРОЗОВА, Е.М., КАРПОВА, Л.В. О содержании ДНК в ядрах мужского гаметофита некоторых цветковых растений. В: *Доклады АН СССР.* 1990. 251. № 4. с. 1023-1024. ISSN: 0869 – 5652.

14. ЖУЧЕНКО, А.А. *Генетика томатов*. Кишинев. 1973. 664 с.
15. ЖУЧЕНКО, А. А. *Мобилизация генетических ресурсов цветковых растений на основе их идентификации и систематизации.* Москва. 2012. 581 с. ISBN: 978-5-85941-452-9.
16. ИГНАТОВА, С.И., БАБАК, О.Г., БАГИРОВА, С.Ф. Создание высоколикопиновых гибридов томата для теплиц с использованием традиционных методов селекции и молекулярных маркеров. В: *Овощи России*. 2020. № 5. с. 22-28. ISSN: 2072 – 9146.
17. КИЛЬЧЕВСКИЙ, А.В., АНТРОПЕНКО, Н.Ю., ПУГАЧЕВА, И.Г. Изучение наследования урожайности и холодостойкости томата (*Lycopersicon esculentum* Mill.) В: *ИНАН Беларуси (Серия Аграрных наук)*. 2007. № 4. с. 68-72. ISSN: 1817 – 7204.
18. КРАВЧЕНКО, А.Н. Методика приготовления цитологических препаратов для их анализа на «Морфокванте» В: *Рекомбиногенез: его значение в эволюции и селекции:* Мат. Всес. конф., 22-24 октября 1985. Кишинев: Штиинца. 1986. с. 264-265.
19. КРАВЧЕНКО, А.Н., ЛЯХ, В.А., ТОДЕРАШ, Л.Г., и др. *Методы гаметной и зиготной селекции томатов.* Кишинев. 1988. 140 с. ISBN: 5-376-00414-7.
20. КРАВЧЕНКО, В.А. Гетерозис в селекции томата. В: *Сборник науч. тр. по прикладной ботанике, генетике и селекции.* Ленинград: ВИР. 1991. Т. 145. с. 37-40. ISSN: 0202 – 3628.
21. КУЗЕМИНСКИЙ, А.В**.** *Селекционно-генетические исследования мутантных форм томата.* Харьков. 2004, 391 с. ISBN: 966-8431-00-6.
22. ЛЫСКО, И.А. *Особенности функционирования репродуктивной системы видов рода Lycopersicon Tourn:* Дисс. канд. биол. наук. Москва. 2013. 156 с.
23. ЛЯХ, В.А., СОРОКА, А.И. Пыльцевой отбор как способ интенсификации селекции масличных культур. В: *Науково-технічний бюллетень Інституту олійних культур НААНУ*. 2014. № 20. р. 72-80. ISSN: 2078 – 7316.
24. **МАКОВЕЙ, М.Д**. Изучение наследования устойчивости к температурному стрессу у гибридов F_1 по признакам мужского гаметофита. В: *Известия Академии наук Молдовы. Науки о жизни.* 2008. № 3 (306). с. 57 - 66. ISSN: 1857 – 064X.
25. **МАКОВЕЙ, М.Д**., КРАВЧЕНКО, А.Н. Анализ гибридов F_2 томата по признакам мужского гаметофита и отбор лучших рекомбинантов. В: *Современные тенденции в селекции и семеноводстве овощных культур.* Межд. науч-практ. конф. Москва. 2008. Т. 2. с. 171-175.
26. **МАКОВЕЙ, М.Д**., ИГНАТОВА, С.И. Характер проявления адаптивности по признакам мужского гаметофита томата к температурному стрессу при выращивании растений в разные годы. В: *Современные тенденции в селекции и семеноводстве овощных культур.* II Межд. научн. - практ. конф. Москва. 2010. Т.1. с. 391 - 400. ISBN: 978-5-901695-42-5.
27. **МАКОВЕЙ, М.Д.** Селекционная ценность гибридных популяций томата, полученных из трансгрессивных рекомбинантов устойчивых к температурному стрессу. В*: Известия Академии наук Молдовы. Науки о жизни.* 2010. № 1 (310). с. 53 - 61. ISSN: 1857 – 064X.

28. **МАКОВЕЙ, М.** Д. Доля влияния родительских компонентов скрещивания на наследуемость устойчивости к температурным стрессам у томатов. В: *Известия Академии наук Молдовы, Науки о жизни.* 2011. № 1 (313). с. 88-94. ISSN: 1857 – 064X.
29. **МАКОВЕЙ, М.Д**. *Селекция томата на устойчивость к стрессовым абиотическим факторам с использованием гаметных технологий.* Кишинев. 2018. 473 с. ISBN: 978-9975-56-565-3.
30. **МАКОВЕЙ, М.Д.** *Потенциал мутантных форм томата для селекционно-генетических исследований.* Tipografia "Print-Caro" 2022. 208 p. ISBN 978-9975-165-22-8.
31. УЗУН, И. В. Изучение функциональной мужской стерильности у томата и методы ее определения В: *Селекция на адаптивность и создание нового генофонда в современном овощеводстве (VI Квасниковские чтения).* Межд. научно-практ. конф. ВНИИО. Москва. 2013. с. 321-324. ISBN: 978-5-902946-19-9.
32. ЧЕСНОКОВ, Ю.В., КОСОЛАПОВ, В.М., САВЧЕНКО, И.В. Морфологические генетические маркеры у растений. В: *Генетика*. 2020. Т. 56. № 12. с. 1366-1377. ISSN: 0016 – 6758.
33. ШТУББЕ, Г. О связях между естественным и искусственно полученным многообразием мутантных форм и о некоторых экспериментальных исследованиях по эволюции культурных растений. В: *Генетика.* 1966. № 11. с. 9-30. ISSN: 0016 – 6758.
34. ШУЛАЕВ, В.К. *Структура хроматина клеток мужского гаметофита в связи с их функциональной активностью.* Автореф. дисс. к. б. н. Кишинев. 1985. 19 с.
35. ANOKHINA, V. The use of gametophytic selection for differentiation of lupin tolerance to stress. /V. Anokhina, V. Duksina, I Sauk. In: *Proceeding of the 13th International Confer. Lupin.* Poznan, Polan. 6-10 June 2011. pp. 285-287. ISBN: 978-605-9831-95-6.
36. AtlasBig. com (2018-2020). https://www.atlasbig.com/en-ie/countries-by-tomato-production
37. BIRADAR, D.P., RAYBURN, A. L. Heterosis and nuclear DNA content in maize In: *Heredity*. 1993. Vol. 71, No 3. pp. 300-304. ISSN: 0018 – 067X.
38. CATALOGUL SOIURILOR de PLANTE a REPUBLICII MOLDOVA *pentru anii* – 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2022. Chișinău.
39. CONG, B., TANKSLEY, S.D. FW2.2 and cell cycle control in developing tomato fruit: a possible example of gene co-option in the evolution of a novel organ. In: *Plant Molecular Biologic.* 2006. 62. pp. 867-880. ISSN: 0167 – 4412.
40. DE STORME, N, GEELEN, D. The impact of environmental stress on male reproductive development in plants: biological processes and molecular mechanisms. In: *Plant Cell Environ*. 2014. No 37(1). pp. 1-18. ISSN: 1532 – 298X (web).
41. DOMINGUES, E. Breeding tomato for pollen tolerance to low temperatures by gametophytic selection /Domingues, J. Cuartero, R. Fernandez-Munoz In: *Euphytica.* 2005. Vol. 142. Iss. 3. pp. 253- 263. ISSN: 1573 – 5060.

42. KANG, M.S. Breeding: Genotype-by-environment interaction. In: R.M. Goodman (ed.). *Encyclopedia of Plant and Crop Science.* New York: Marcel-Dekker; 2004. pp. 218-221. ISBN: 0491334386.
43. KIMURA, S. Tomato (*Solanum lycopersicum*): A Model Fruit-bearing Crop. In: *Emerging Model Organisms:* Laboratory Manual. New York: Cold Spring Harbor Laboratory Press. 2008. Vol. 1. pp. 119-138. ISBN: 978-0879698720.
44. KHOTYLEVA, L.V., KILCHEVSKY, A.V., SHAPTURENKO, M.N. Theoretical aspects of heterosis. In: *Vavilov Journal of Genetics and Breeding*. 2016. №. 20(4). pp. 482-492. ISSN: 2500 - 0462.
45. LUSSER, M., PARISI, C., PLAN, D., RODRIGUEZ-CEREZO, E. Deployment of new biotechnologies in plant breeding. In: *Nature biotechnology.* 2012. No 30. pp. 231-239. ISSN: 1087 – 0156.
46. LYAKH, V.A. Influence of pollen heating on the qyality of resulting sporophyte generation in sunflower /V.A. Lyakh, N. V. Gasenko, A.I. Soroka. In: *Helia*. 1998. Vol. 21. Iss. 29. pp. 103-108. ISSN: 2197 – 0483.
47. IGNATOVA, S., **MAKOVEI, M**. Development and use of abiotic stress tolerance coefficient in tomato breeding. In: *XVIIIth EUKARPIA Meeting on Genetics and Breeding on Tomato. Vegetable Section. Tomato Working* - Abstracts. Avignon 2014. pp. 22-25.
48. MAISONNEUVE, B., HOGENBOOM, N.G., DEN NIJS A.P.M. Pollen selection in breeding tomato (*Lycopersicon esculentum* Mill.) for adaption to low temperature. In: *Euphytica*. 1986. Vol.35. No. 3. pp. 983-992. ISSN: 1573 – 5060.
49. **MAKOVEI M.** Impact of the term of storage of tomato pollen at low temperature on its quality. In: *XIth International Congress of Geneticists and Breeders from the Republic of Moldova. Abstract Book.* Chisinau, 2021, p. 98. ISBN: 978-9975-933-56-8.
50. MASCARENHAS, G.P., MITCHEL ALTSHULER. The response of pollen to high-temperatures and its potential applications. In: *Pollen: Biology and Implications for Plant Breeding.* (Ed. Mulcahy D.L., Ottaviano Ercole). New-York. 1982. P. 3-8. ISBN-10: ISBN: 978-0444007384.
51. MULCAHY, D.L., MULCAHY, G.B., ROBINSON, R.W. Evidence for postmeiotic genetic activity in pollen og Cucurbita Species. In: *J. of Heredity*. 1979. V.70. No. 6. pp. 365-368. ISSN: 0022–1503.
52. MORISHIMA, H. Inheritance of low temperature resistance at the young seedling stage in rice. In: *Annual Report of the National Instute of Genetics*. Jap. 1991, No. 42. pp. 103-104. ISSN: 0077–4995.
53. OTTAVIANO, E., SARI-GORLA, M. Gametophytic and sporophytic selection. In: *Plant Breeding Principles and Prospects*. Chapman and Hall, 1993. pp. 333-352. ISBN: 978-0412433900.
54. PFAHLER, P.L. Comparative effectiveness of pollen genotype selection in Higher plants. In: *Polen: Biology and Implications for Plant Breeding*. N.Y. 1982. pp. 361-366. ISBN: 978-0444007384.
55. RAIGON, D. Eggplant relatives as sources of variation for developing new rootstocks: Effects of grafting on eggplant yield and fruit apparent quality and

composition. In: *Scietia Horticulturae*. 2011. Vol. 128, Issue 1. pp. 112-119. ISSN: 0304 – 4238.

56. RAUBURN, A. L, BIRADAR, D. P., BULLOCK, D. G.T., McMURPHY, I. M. Nuclear DNA content in F_1, hybrids of Maize In: *Heredity*. 1993. V.71, No 3. pp. 294-300. ISSN: 0018 – 067X.
57. SALEM, MA, KAKANI, VG, KOTI, S, REDDY, KR. Pollen-based screening of soybean genotypes for high temperatures. In: *Crop Science*. 2007. No 47. pp. 219-231. ISSN: 1435 – 0653.
58. SALTANOVICI, T.; ANTOCI, L. Evidențierea genotipurilor de tomate rezistente la stresul hidric prin analiza grăuncioarelor de polen. *Mat. conf. șt. intern. (ediția a VI-a) «Genetica, fiziologia și ameliorarea plantelor»*. Chișinău. 2017. pp. 68-71. ISBN: 978-9975-56463-2.
59. TANKSLEY, S.D., MUTSCHLER, M.A. Linkage map of the tomato (*Lycopersicon esculentum* L). In: *Genetic maps*. 1989. pp. 6.3 - 6.15. ISSN: 0738 – 5269.
60. TANKSLEY, S.D. The genetic, developmental and molecular bases of fruit size and shape variation in tomato. In: *The Plant Cell*. 2004. Vol. 16. pp. 181-189. ISSN: 1040 – 4651.
61. WANN, E.V., JORDAN, E.L., PRESSEY, R., LYON, B.G. Efect of mutant genotype *hp, og*^{c} and *dg, og*^{c} on tomato fruit quality. In: *J. Amer. Hort. Sci*. 1985. V. 110. pp. 212-215. ISSN: 0003 – 1062.

LIST OF PUBLICATIONS ON THE TOPIC OF THE DISSERTATION

1. Specialty books
1.1. Single-author specialty books

MAKOVEI, M.D. Tomato selection for resistance to abiotic stress factors using gamete technologies. Chisinau: Print-Caro, 2018. 473 p. ISBN 978-9975-56-565-3 (Ru).

MAKOVEI, M.D. Morphobiological basis of tomato cultivation. Chisinau: Print-Caro, 2021. 156 p. ISBN 978-9975-56-841-8 (Ru).

MAKOVEI, M.D. Potential of mutant forms of tomato for breeding and genetic research. Chisinau: Print-Caro, 2022. 208 p. ISBN 978-9975-165-22-8 (Ru).

2. Articles in scientific journals
2.1. in international journals listed in SCOPUS

MAKOVEI, M.D., KRAVCHENKO, A.N., IGNATOVA, S.I. Variability of DNA content and chromatin dispersion in the nuclei of pollen generative cells under the influence of temperature. In: *Agricultural Biology*. Moscow. 2001. No. 3. p. 67-72. ISSN 0131 – 6397 (Ru).

MAKOVEI Milania, LUPASCU Galina. Formation recombination of variability in tomato F_2 population with the participation mutant marker genes. In: *Bulgarian Journal of Agricultural Science* (expediat pe 31 may 2023).

MAKOVEI Milania, LUPASCU Galina. Variability of morphological traits of tomato vegetative and generative pollen cell nuclei in lines and F_1 hybrids under stress temperatures. In: *Acta Agriculturae Slovenica* (expediat pe 25 iulie 2023, nr. registrar 15085).

2.2. In recognized foreign scientific journals

MAKOVEI, M.D., IGNATOVA, S.I. Microgametophytic selection in tomato breeding for stress resistance. In: *Potatoes and vegetables*. Moscow. 2010. No. 1. p. 27-28. ISSN: 0022 –9148 (Ru).

SÎROMEATNICOV, I., COTENCO, E., CIOBANU, R., JACOTĂ, A., ŞVEŢ, Ş., **MAKOVEI, M**. The efficiency of natural bioregulators in the selection of valuable tomato genotypes. In: *Muzeul Olteniei Craiova. Oltenia* Studii şi communicări. Ştiinţele Naturii. 2010. T. 26. N.1. pp. 19-24. ISSN: 1454 + 6914 (Ro).

MAKOVEI, M. D. Intrapopulation variability in F_3 and F_4 progeny of tomato obtained from F_2 recombinants resistant to high temperature according to the characteristics of the male gametophyte. In: *Vegetables of Russia*. Moscow. 2019. No. 4. p. 37-43. ISSN: 2072 –9146 (Ru).

MAKOVEI, M. D. Selection method and its influence on the intensification of the selection process. In: *Vegetables of Russia*. Moscow. 2020. No. 5. p. 21-25. SSN: 2072 – 9146 (Ru).

MAKOVEI M.D. The nature of the impact of mutant genes on the manifestation of selection-valuable traits in tomato. In: *Agrophysics.* St. Petersburg, 2022. No. 2. p. 13-20. ISSN: 2222 – 0666. DOI: 10.25695/AGRPH.2022.02.03 (Ru).

2.3. In specialized journals from the National Register indicating the category

MAKOVEI, M.D. The influence of pollination with preheated pollen on the resistance of F_1 tomato hybrids. In: *Journal of Academy of Sciences of Moldova. Biological, chemical and agricultural sciences*. 2003. No. 2. p. 94-97. ISSN: 1857–064X (Cat. B) (Ru).

MAKOVEI, M. D. Study of the inheritance of resistance to temperature stress in F_1 hybrids according to the characteristics of the male gametophyte. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2008, nr. 3(306), pp. 57-66. ISSN: 1857–064X (Cat. B) (Ru).

MAKOVEI, M., GRATI, M., MIKHNYA, N., GRATI, V. Screening of the cultivated tomato gene pool for resistance to high temperature stress based on pollen traits. In: *Agricultural Science,* 2009, nr. 2, pp. 18-22. ISSN: 1857–0003 (Cat. B) (Ru).

MIHNEA, N., GRATI, M., **MAKOVEI, M.,** JACOTĂ, A. The heat resistance genetic potential of valuable tomato varieties and lines. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2009, no. 2(308), pp. 64-69. ISSN: 1857–064X (Cat. B) (Ro).

MAKOVEI, M. D. Breeding value of hybrid populations of tomatoes, obtained from transgressive recombinants that are resistant to pollen traits. In: *Journal of*

Academy of Sciences of Moldova. Life sciences, 2010. N.1 (310). pp. 53-61. ISSN: 1857 – 064X (Cat. B) (Ru).

MAKOVEI, M.D. The share of the influence of parental components of crossing on the heritability of resistance to temperature stress in tomatoes. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2011, N.1 (313), pp. 88-94. ISSN: 1857 – 064X (Cat. B) (Ru).

MAKOVEI, M.D. Genetic heterogeneity of mutant forms of tomato in terms of resistance of the male gametophyte to heat, drought and cold. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2012, N. 2 (317), pp. 76-84. ISSN: 1857 – 064X (Cat. B) (Ru).

MAKOVEI, M.D., GANYA, A.I. Mutant forms of tomatoes as sources of economically valuable traits for breeding. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2012, N. 2 (317), pp. 91-100. ISSN: 1857 – 064X (Cat. B) (Ru).

MAKOVEI, M.D. Current trends in tomato breeding. In: *Agriculture of Moldova*. 2014. N. 3-4, p. 34-37. ISSN 0582 5229 (Cat. C) (Ru).

MAKOVEI, M.D., GANYA, A.I. Genetic potential of a collection of tomato mutants and its significance for solving practical breeding problems. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2015. N. 1(325), pp. 110-119. ISSN: 1857 – 064X (Cat. B) (Ru).

MAKOVEI, M.D. Genotypic variability in the main selection-valuable traits in the progeny of a tomato varietal hybrid. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2016. N. 2 (329), pp. 61-69. ISSN: 1857 – 064X (Cat. B) (Ru).

MAKOVEI, M.D., BOTNARI, V.F. New tomato varieties with a complex of economically valuable traits for cultivation in Moldova. In: *Agricultural Science,* 2016. N. 1. pp. 60-65. ISSN: 1857 – 0003 (Cat. B) (Ru).

MAKOVEI, M.D., BOTNARI, V.F. Prediction of the efficiency of selection of initial parental forms when creating heterotic F_1 tomato hybrids by determining the degree of dominance of selection-valuable traits. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2017. N.1 (331), pp. 73-82. ISSN: 1857–064X (Cat. B) (Ru).

MAKOVEI, M.D., BOTNARI, V.F. Creation of tomato source material with functional sterility for use in heterotic breeding. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2017. N. 2 (332), pp. 76-85. ISSN: 1857 – 064X (Cat. B) (Ru).

MAKOVEI, M.D. Variability of male gametophyte traits in promising tomato lines depending on the level of inflorescence initiation on the vegetative plant. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2017. N. 3 (333), pp. 96-106. ISSN: 1857–064X (Cat. B).

MAKOVEI, M.D. Comparative analysis of the effectiveness of using a combination of traditional and non-traditional selection methods in tomato breeding. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2018. N.3 (336), pp. 115-124. ISSN: 1857 – 064X (Cat. B).

MAKOVEI, M.D. Variability of some pollen traits in mutant forms of tomato under the influence of abiotic stress. In: *Journal of Academy of Sciences of Moldova. Life sciences,* 2019. N. 1 (337), pp. 81-91. ISSN: 1857 – 064X (Cat. B) (Ru).

3. Articles in collections of materials of international and national scientific conferences

MAKOVEY, Milania. Creation of sterile forms of tomato for heterotic selection. In: *International Congress of Geneticists and Breeders from the Republic of Moldova*, Ediția 10, 28 June 1 July 2015, Chisinau, pp. 153-154. ISBN 978-9975-4126-1-2.

MAKOVEY, Milania. Phenotypic variability of yield subcomponents in mutant forms of tomato. In: *"Genetics, physiology and plant breeding: international scientific conference",* Ediţion 6, 23-24 October 2017, Chişinău. Chişinău: Tipogr. "PrintCaro", 2017, pp. 132-136. ISBN 978-9975-56-463-2.

MAKOVEY, Milania. Genotypic features of tomato lines carrying mutant marker genes. In: *Horticulture, Viticulture and winemaking, Forestry and public gardens, Plant protection: International Scientific Symposium "Modern horticulture - achievements and perspectives"*, 1-2 October 2018, Chișinău, Republica Moldova: Universitatea Agrară de Stat din Moldova, 2018, Vol. 47, pp. 137-142. ISBN 978-9975-64-296-5.

MAKOVEY, Milania. The selection method and its influence on obtaining heat-resistant tomato offspring at different stages of ontogenesis. In: *Horticulture, Viticulture and winemaking, Forestry and public gardens, Plant protection: International Scientific Symposium "Modern horticulture - achievements and perspectives"*, 1-2 Octomber 2018, Chișinău, Republica Moldova: Universitatea Agrară de Stat din Moldova, 2018, Vol. 47, pp. 142-148. ISBN 978-9975-64-296-5.

MAKOVEY, Milania. Peculiar features of the inheritance of the trait vegetation duration by F_1 hybrids derived from crossing of *RIN* and *NOR* mutant gene forms. In: *Advanced biotechnologies – achievements and perspectives: The national scientific symposium with international participation,* Edition 5, 21-22 October 2019, Chisinau, Republic of Moldova: Centrul Editorial-Poligrafic al USM, 2019, p. 163. ISBN 978-9975-56-695-7.

MAKOVEY, Milania. Priority directions in tomato breeding and genotypic characteristics of varieties intended for cultivation in Moldova. In: *Science, education, culture,* February 15, 2020, Comrat, Republic of Moldova: State University of Comrat, 2020, Vol.1, pp. 388-395. ISBN 978-9975-83-091-1.

MAKOVEY, Milania. Selection for heterosis and frequency of repetition of types of inheritance of the main economically valuable traits of tomato by F_1 hybrids. In: *Science in the North of the Republic of Moldova: achievements, problems, perspectives,* Edition 4, June 26-27, 2019, Balti, Republic of Moldova: Tipogr. "Indigo Color", 2020, pp.73-78. ISBN 978-9975-3382-6-4.

MAKOVEY, Milania. Impact of the term of storage of tomato pollen at low temperature on its quality. In: *International Congress of Geneticists and Breeders from the Republic of Moldova*, Edition 11, June 15-16, 2021, Chisinau, Republic of Moldova: Editorial-Polygraphic Center of the State University of Moldova, 2021, p. 98. ISBN 978-9975-933-56-8.

MAKOVEY, Milania. Use of mutant tomato genes in breeding for heterosis. In: *Genetics, physiology and plant breeding,* Edition 7, October 4-5, 2021, Chisinau: Tipogr. "PrintCaro", 2021, pp. 229-232. ISBN 978-9975-56-912-5.

MAKOVEY, Milania, GANYA, Anatoly. Heterogeneity of the mutant tomato collection by growth type and plant habit. and heterosis. In: *Genetics, physiology and plant breeding,* Edition 7, October 4-5, 2021, Chisinau: Tipogr. "PrintCaro", 2021, pp. 233-236. ISBN 978-9975-56-912-5.

MAKOVEY, Milania. The influence of the level of inflorescence initiation on the parameters of tomato pollen and its resistance to high temperature. In: *Science in the North of the Republic of Moldova: achievements, problems, perspectives,* Edition 6, May 20-21, 2022, Balți. Balti, Republic of Moldova, 2022, pp. 84-87. ISBN 978-9975-81-128-6.

MAKOVEY, Milania. The splitting of traits controlled by *Ls* and *Br* genes in F_2 populations of tomato. In: *Advanced biotechnologies – achievements and perspectives,* Edition 6, October 3-4, 2022, Chisinau, Republic of Moldova: USM Editorial-Polygraphic Center, 2022, pp. 306-308. ISBN 978-9975-159-81-4.

MAKOVEY, Milania. Phenotypical variability of fetal traits in mutant tomato forms. In: *Life sciences in the dialogue of generations: connections between universities, academia and business community*, Ediția 2, 29-30 septembrie 2022, Chişinău. Republica Moldova: Moldova State University, 2022, R, p. 47. ISBN 978-9975-159-80-7.

MAKOVEY, Milania. Using mutant *ls* and *br* genes of tomato to create a new sourse material. In: *Life sciences in the dialogue of generations: connections between universities, academia and business community*, Ed. 2, 29-30 septembrie 2022, Chişinău. Chișinău, Republica Moldova: Moldova State University, 2022, p. 48. ISBN 978-9975-159-80-7.

MAKOVEY, Milania. Mutant forms of tomato and their significance for breeding and genetic research. In: *Science in the North of the Republic of Moldova: achievements, problems, perspectives,* Edition 7, May 19-20, 2023, Balti. Balti, Republic of Moldova: Bons Offices, 2023, pp. 88-93. ISBN 978-9975-81-128-6.

MAKOVEI, Milania. The genotypic reaction of pollen of mutant tomato forms, to the action of stressful abiotic factors**.** Second International Scientific Conference *«Plants Stress and Adaptation»* dedicated to the 125th anniversary of the birth of Prof. F.P. Matskov Kharkov, June 7-8, 2023. P. 138-139.

MAKOVEI, Milania. Mutant genes controlling traits of the reproductive system of tomato. The National Conference with international participation *Natural sciences in the dialog of generation.* The conference will be held in Chisinau, on 14-15 September 2023 *ABSTRACT BOOK***.** P. 50. ISBN 978-9975-3430-9-1.

Plant variety patents

MIHNEA, N., GRATI, V., LUPAŞCU, G., BOTNARI, V., **MAKOVEI, M.** Patent for a plant variety. TOMATOES (*S. lycopersicum* L.) Variety *MARY GRATEFYLLY*. MD 149 din 2014.01.31. In: *BOPI* nr. 5, 2014, p. 42. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M., GUSEVA, L., BELEAEV, P., BOTNARI, V., LUPAŞCU, G. Patent for a plant variety. TOMATOES (*S. lycopersicum* L.) Variety *MAKRISTA*. MD 157 din 2014.06.30. In: *BOPI* nr. 6, 2014, p. 46. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M., GUSEVA, L., BELEAEV, P., BOTNARI, V. Patent for a plant variety. TOMATOES (*S. lycopersicum* L.) Variety *MILORANJ*. MD 158 din 2014.06.30. In: *BOPI,* nr. 6, 2014, p. 46. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M., GUSEVA, L., BELEAEV, P., BOTNARI, V., ŞTEFÎRŢĂ, A. Patent for a plant variety. TOMATOES (*S. lycopersicum* L.) Variety *STEFANI*. MD 159 din 2014.06.30. In: *BOPI*, nr. 4, 2014, p. 46. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M., GUSEVA, L., BOTNARI, V. Patent for a plant variety. TOMATOES (*S. lycopersicum* L.) Variety *PRICHINDEL*. MD 242 din 2017.08.31. In: *BOPI,* nr. 4, 2017. p. 48. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M., GUSEVA, L., BOTNARI, V. Patent for a plant variety. TOMATOES (*S. lycopersicum* L.) Variety *VIVAT*. MD 243 din 2017.08.31. In: *BOPI,* nr. 4, 2017, p. 49. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M. Patent for a plant variety. TOMATOES (*S. lycopersicum* L.) Variety CIREAȘCĂ. MD 307 din 2019.12.31. In: *BOPI,* nr. 8, 2019, p. 63. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M., BOTNARI, V., CANEA, A. Patent for plant variety. TOMATOES **(***S. lycopersicum* L.). Variety MATRIONA. MD 309 of 2019.12.31. In: BOPI, no. 8, 2019, p. 63. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M., BOTNARI, V. Patent for a plant variety. TOMATOES (*S. lycopersicum* L.) Variety *DIMETRA***.** MD 372 din 2021.05.18. In: *BOPI,* nr. 7, 2021, p. 66. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M., BOTNARI, V. Patent for a plant variety. TOMATOES (*S. lycopersicum* L.) Variety *ILICA***.** MD 375 din 2021.05.18. In: *BOPI*, nr. 7, 2021, p. 66. ISSN: 1810 – 7095 (Ro).

MAKOVEI, M. Patent applications filed for AGEPI. TOMATOES (*S. lycopersicum* L.) Variety *PETRAMAK* – **V 2020 0013 from 24. 07. 2020** (Ro).

MAKOVEI, M., LUPASCU, G., GANEA, A**.** Patent applications filed for AGEPI. TOMATOES (*Solanum lycopersicum* L.) **Hybrid F$_1$** *ROZAMAK* nr. Applications: **575 from 2022. 04. 13** (Ro).

MAKOVEI, M., LUPASCU, G., GANEA, A. Patent applications filed for AGEPI. TOMATOES (*Solanum lycopersicum* L.) **Hybrid F$_1$** *INGSTAR* nr. Applications: **576 from 2022. 04. 13** (Ro).

ANNOTATION

MAKOVEI Milania, " Genetic potential of the cultivated and mutant gene pool of tomato (S*olanum lycopersicum* l.), methods of research and use in breeding

Doctor habilitatus thesis in biological sciences, Chisinau, 2023

Structure of the thesis: introduction, 6 chapters, general conclusions and recommendations; the bibliography includes 437 titles, the thesis is completed with 10 annexes, has 256 text pages, 49 tables, 44 figures. The results are published in 172 scientific works.

Research area: Plant breeding and seed production.

Key words: tomato, gene pool, breeding, resistance, microgametophyte, abiotic stress factors, variability, heritability, mutant genes, heterosis, lines, varieties.

The aim of the research: identification of the genetic potential of tomato cultivars and mutants at the gametophytic and sporophytic level by analyzing the variability and heritability of the resistance to unfavorable abiotic factors and other economically valuable traits in order to develop new initial material, high performance varieties and hybrids.

Study objectives: development and optimization of artificial stress backgrounds (high, low temperatures and drought) for resistance *screening* of mutant forms and other tomato genotypes based on the traits of the male gametophyte under *in vitro* conditions; studies on the genetic potential of the gene pool of cultivated and mutant tomatoes as a new source of germplasm to enhance the resistance to abiotic stress factors, with the simultaneous assessment of the manifestation of biological, economically valuable, and mutant marker traits; carrying out crosses between several parents including the use of mutant genes to produce new hybrid populations; studies on the variability and inheritance by F_1 hybrids of the biological, cytochemical, and other traits of the pollen that determine the resistance to abiotic stress factors for the subsequent optimization of the selection procedures of the parents and production of resistant genotypes; estimation of the rate of the influence of parental forms and their interaction on the heritability of pollen heat and cold resistance traits in F_1; development of the selection method of resistant transgressive recombinants from F_2 populations based on the traits of the male gametophyte; development of combined selection methods simultaneously employing traditional ones and gametic technologies; development of tomato initial material with mutant marker traits to be used in heterotic breeding; development of tomato varieties and hybrids with high producing capacity and resistance to abiotic stress factors that combine economically valuable traits, including mutant marker ones.

Scientific novelty and originality: for the first time, a collection of tomato single- and multi-marker mutant forms has been evaluated for the resistance to abiotic stress factors (high, low temperatures, drought) based on male gametophyte traits. The resistance to above factors has been shown to be determined by different genetic systems. Have been: (i) elucidated the particular features of variability and heritability of cytochemical, biological, and economically valuable traits depending on genotype and environmental conditions; (ii) developed of methodology to make the tomato breeding process more efficient by combining gametic and classical selection approaches.

Principal results for science and practice. (i) New generation initial material with different combination of resistance to three abiotic stress factors (high and low temperatures, and drought), including complexes of economically valuable traits and mutant marker properties. (ii) The importance of combined employment of gametic and classical breeding methods has been scientifically substantiated. (iii) Varieties (11), heterotic hybrids (2), and improved tomato lines have been developed, that are diverse genetically (>50).

Theoretical significance. (i) The limits of variability and heritability of biological and cytochemical traits and some marker properties of mutants under the influence of high and low temperatures have been estimated, which allowed the assessment of adaptability level in tomato genotypes and the optimization of parental form selection for crossings. (ii) The rate of parental influence on drought and cold resistance in F_1 hybrids has been demonstrated. (iii) The peculiar features of transgressive variability in F_2 populations have been found, allowing early prediction of the selection efficiency of resistant recombinants and the breeding value of the offspring in F_3-F_4.

Application value. Tomato hybrid populations (more than 140) with a wide range of variability of economically valuable traits have been developed. F_1 heterotic varieties and hybrids of tomato which differ in the growth type, plant architectonics, shape, color, fruit weight, and diverse combination of mutant marker traits – *dw, ssp, sp,* $sp^{\pm}$, sp^{+}, *rin, nor, ex, ps, hp, ls, br, j-2,* etc. have been developed for open and protected soil.

Implementation of scientific results. The newly developed tomato initial material, varieties, and F_1 hybrids have been included in the breeding programs of some laboratories of the Institute of Genetics, Physiology and Plant Protection of the State University of the Republic of Moldova and in the Russian Federation to produce new forms of tomatoes; they are still used in the working collections of the Plant Genetic Resources Laboratory (IGFPP, USM) and are cultivated in various individual households and by farmers.

АННОТАЦИЯ

МАКОВЕЙ Миланья Дмитриевна, «Генетический потенциал культурного и мутантного генофондов томата (*Solanum lycopersicum* L.), методы исследования и использования в селекции».
Диссертация на соискание ученой степени доктора хабилитат биологических наук,
Кишинев, 2023

Структура диссертации: введение, 6 глав, общие выводы и рекомендации, список литературы из 437 наименований, 10 приложений, 256 страниц основного текста, 49 таблиц, 44 рисунка. Полученные результаты опубликованы в 172 научных работах.

Область исследования: Селекция растений и семеноводство.

Ключевые слова: томат, генофонд, селекция, устойчивость, мужской гаметофит, абиотические стресс-факторы, изменчивость, наследование, мутантные гены, гетерозис, линии, сорта.

Цель исследования: выявление генетического потенциала культурных и мутантных форм томата на гаметофитном и спорофитном уровне путем анализа изменчивости и наследуемости устойчивости к стрессовым абиотическим факторам и других хозяйственно-ценных признаков для создания нового исходного материала, высокопродуктивных сортов и гибридов.

Задачи: создать и оптимизировать искусственные стрессовые фона (высокие, низкие температуры, засуха) для *скрининга* мутантных и других генотипов томата по признакам мужского гаметофита в условиях *in vitro;* изучить генетический потенциал культурного и мутантного генофондов томата как источника новой зародышевой плазмы на устойчивость к абиотическим стрессам с одновременной оценкой проявления биологических, хозяйственно-ценных и мутантных маркерных признаков; получить новые гибридные популяции; изучить изменчивость и наследование гибридами F_1 биологических, цитохимических и других признаков пыльцы, детерминирующих устойчивость к стресс-факторам для последующей оптимизации способов эффективного подбора родительских пар и получения устойчивых генотипов; определить долю влияния родительских форм и их взаимодействия на наследуемость признаков жаро- и холодостойкость пыльцы гибридами F_1; разработать метод отбора устойчивых трансгрессивных рекомбинантов из популяций F_2 по признакам мужского гаметофита; разработать методы комбинированных отборов в сочетании традиционной селекции с гаметными технологиями; создать исходный материал с мутантными маркерными признаками для гетерозисной селекции; создать высокопродуктивные и устойчивые к стрессовым абиотическим факторам сорта и гибриды томата с разным сочетанием хозяйственно-ценных признаков, включая мутантные маркерные.

Научная новизна и оригинальность работы: впервые изучена коллекция одно- и многомаркерных мутантных форм томата на устойчивость к абиотическим стрессам (высокая, низкая температуры, засуха) по признакам мужского гаметофита. Показано, что устойчивость к этим стресс-факторам детерминируется разными генетическими системами. Выявлены: 1) особенности изменчивости и закономерности наследования цитохимических, биологических и хозяйственно-ценных признаков томата гибридами F_1 в зависимости от генотипа и условий среды; 2) разработана методология интенсификации селекции при комплексном использовании гаметных и классических подходов.

Основные результаты для науки и практики: 1) получен исходный материал нового поколения с разным сочетанием устойчивости к трем абиотическим стрессорам (высокие, низкие температуры, засуха), включая комплекс хозяйственно-ценных и маркерных признаков; 2) научно обоснована значимость сочетания методов пыльцевой и классической селекции: 3) созданы новые сорта (11), гетерозисные гибриды (2) и генетически разнокачественные селекционные линии томата (>50).

Теоретическая значимость работы. Установлены: 1) лимиты изменчивости и закономерности наследования биологических, цитохимических и некоторых мутантных маркерных признаков под действием высоких и низких температур, позволяющие определить уровень адаптивности генотипов и оптимизировать подбор пар для скрещивания; 2) доля влияния родительских форм на устойчивость к жаре и холоду гибридов F_1; 3) особенности трансгрессивной изменчивости в популяциях F_2 и возможность раннего прогнозирования эффективности отборов устойчивых рекомбинантов и селекционная ценность их потомств в поколениях F_3-F_4.

Прикладная значимость. Получены гибридные популяции (более 140) с широким спектром изменчивости селекционно-ценных признаков. Созданы линии, сорта и гетерозисные гибриды F_1 томата, отличающиеся по типу роста и архитектонике куста, формой, цветом и массой плода, с разным сочетанием маркерных признаков – *dw, ssp, sp,* $sp^{\pm}$, sp^{+}, *rin, nor, ex, ps, hp, ls, br, j-2* и другие.

Внедрение научных результатов. Созданный исходный материал, сорта и гибриды F_1 томата включены в селекционные программы некоторых лабораторий Института генетики, физиологии и защиты растений Государственного Университета Молдовы и Российской Федерации для получения новых форм томата; используются в активных коллекциях Лаборатории генетических ресурсов растений (ИГФиЗР, ГУМ), а также внедрены в различные производственные и фермерские хозяйства.

ADNOTARE

MAKOVEI Milania, "**Potențialul genetic al genofondurilor tomatelor de cultură și mutante (*Solanum lycopersicum* L.), metode de cercetare și utilizare în ameliorare**".

Teză de doctor habilitat în științe biologice, Chișinău, 2023

Structura tezei: introducere, 6 capitole, concluzii generale și recomandări, bibliografia cuprinde 437 titluri, teza este completată cu 10 anexe, are 256 pagini de text, 49 de tabele, 44 figuri. Rezultatele obținute sunt publicate în 172 lucrări științifice.

Domeniul de cercetare: Ameliorarea plantelor și producerea semințelor.

Cuvinte cheie: tomate, genofond, ameliorare, rezistență, microgametofit factori abiotici de stres, variabilitate, heritabilitate, gene mutante, heterozis, linii, soiuri.

Scopul cercetărilor: evidenţierea potenţialului genetic al cultivarelor de tomate și a mutanților la nivel gametofitic și sporofitic, prin analiza variabilității și heritabilității rezistenței la factori abiotici nefavorabili și altor caracteristici agronomic valoroase în vederea creării materialului inițial nou, a soiurilor și a hibrizilor performanți.

Obiectivele studiului: crearea și optimizarea fondurilor artificiale de stres (temperaturi ridicate, scăzute, secetă) pentru *screening*-ul rezistenței formelor mutante și altor genotipuri de tomate pe baza caracterelor gametofitului mascul în condiții de *in vitro*; cercetarea potențialului genetic al genofondului tomatelor de cultură și a celor mutante în calitate de sursă nouă de germoplasmă pentru rezistență la factori abiotici de stres, cu estimarea concomitentă a manifestării caracterelor biologice, agronomic valoroase și marker mutanţi; efectuarea încrucișărilor între mai mulţi părinţi, inclusiv cu utilizarea genelor mutante, pentru crearea populațiilor hibride; studiul variabilității și heritabilității caracterelor biologice, citochimice și altor caractere al polenului la hibrizii F_1 care determină rezistența la factorii de stres abiotic pentru optimizarea ulterioară a procedeelor de selecţie a genitorilor și obţinerea genotipurilor rezistente; determinarea ratei de influență a formelor parentale și interacțiunii acestora asupra heritabilității caracterelor de rezistență a polenului la temperatură ridicată și scăzută în generația F_1; elaborarea metodei de selecţie a recombinanților transgresivi rezistenți din populațiile F_2 pe baza caracterelor gametofitului mascul; elaborarea metodelor de selecţie combinată prin asocierea metodelor tradiționale cu tehnologiile gametice; crearea materialului inițial la tomate cu caractere mutante marcate, pentru utilizare în selecția heterotică; crearea soiurilor și hibrizilor de tomate cu producţie ridicată și rezistență la factorii de stres abiotic ce îmbină caractere agronomic valoroase, inclusiv marker mutant.

Noutatea și originalitatea științifică: pentru prima dată a fost studiată o colecție de forme mutante cu un singur marker și multimarker la tomate în vederea decelării rezistenței la factori de stres abiotic (temperaturi ridicate, scăzute, secetă) pe baza caracterelor gametofitului mascul. S-a demonstrat că rezistența la arșiță, frig și secetă este determinată de diferite sisteme genetice. Au fost: i) elucidate particularitățile variabilității și legitățile heritabilității caracterelor citochimice, biologice și agronomic valoroase, în dependență de genotip și condițiile de mediu; ii) elaborată metodologia de eficientizare a procesului de ameliorare la tomate prin asocierea procedeelor de selecţie gametică și clasică.

Rezultatele principiale noi pentru știință și practică. A fost: i) obținut material inițial de nouă generație cu asociere diferită a rezistenței la trei factori de stres abiotic (temperaturi ridicate și scăzute, şi secetă), inclusiv a complexelor de caractere agronomic valoroase și însușiri marker al mutanților; ii) este argumentată științific importanța utilizării asociate a metodelor de selecţie gametică și clasică: iii) sunt create soiuri (11), hibrizi heterotici (2) și linii ameliorate de tomate, diverse din punct de vedere genetic (>50).

Semnificația teoretică. Au fost: i) stabilite limitele variabilității și legitățile heritabilității caracterelor biologice, citochimice și ale unor însușiri marker al mutanților sub influența temperaturilor ridicate și scăzute, ceea ce au făcut posibilă stabilirea nivelului de adaptibilitate a genotipurilor de tomate și s-a optimizat selecția formelor parentale pentru încrucișare; ii) demonstrată rata de influență a formelor parentale asupra rezistenței hibrizilor F_1 la arșiță și la frig; iii) elucidate particularitățile variabilității transgresive în populațiile F_2, fiind demonstrată posibilitatea de prevedere timpurie a eficienței de selecţie a recombinanților rezistenți și valoarea ameliorativă a descendenților în generațiile F_3-F_4.

Valoarea aplicativă. Au fost obținute populații hibride de tomate (mai mult de 140) cu un spectru larg al variabilității caracterelor agronomic valoroase. Au fost create soiuri și hibrizi heterotici F_1 de tomate pentru teren deschis și protejat, ce se evidențiază prin tipul de creștere, arhitectura plantei, forma, culoarea, greutatea fructului și asociere diversă a caracterelor marker al mutanților – *dw, ssp, sp*, $sp^{\pm}$, sp^{+}, *rin, nor, ex, ps, hp, ls, br, j-2* etc.

Implementarea rezultatelor științifice. Materialul inițial, soiurile și hibrizii F_1 de tomate nou create, au fost incluse în programele de ameliorare ale unor laboratoare ale Institutului de Genetică, Fiziologie și Protecție a Plantelor din cadrul Universității de Stat a Republicii Moldova și în Federația Rusă, pentru obținerea unor noi forme de tomate; se mai folosesc în colecțiile de lucru ale Laboratorului Resurse Genetice Vegetale (IGFPP, USM) și sunt cultivate în diverse gospodării individuale și de fermieri.

MILANIA MAKOVEI

GENETIC POTENTIAL OF CULTIVATED AND MUTANT GENE POOL OF TOMATO (*Solanum lycopersicum* L.), METHODS OF RESEARCH AND USE IN BREEDING

411.04. Plant breeding and seed production

Abstract of the thesis for an scientific degree

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