

MARKER TRAITS AND THEIR MANIFESTATION IN THE EARLY STAGES OF TOMATO PLANT DEVELOPMENT

MAKOVEI MILANIA DUMITRU¹

Abstract. The article presents the results of the assessment of the phenotypic expression of mutant marker genes controlling anthocyanin synthesis and the degree of distortion of the shape, color, and first true leaves in tomatoes. Mutant forms (125 genotypes) are classified into groups depending on the nature of manifestation and the degree of phenotypic expression of traits depending on the genotype (single- and multi-marker mutants) and plant growing conditions (field, greenhouse). It has been shown that a higher degree of trait variability is characteristic of multi-marker mutant forms. Single- and multi-marker mutants have been identified in which the marker traits controlled by the genes *afl*, *apn*, *atv*, *aut*, *c*, *cy*, *cla*, *clau* *Cu*, *div*, *ga*, *inta*, *Ln*, *ltf*, *lur*, *lut*, *l-2*, *nv*, *Tor* *Wo^m*, *yg-2*, are phenotypically clearly manifested and easily identified under different growing conditions of their plants (V = 2.4...8.3%).

Keywords: Tomato, mutant forms, marker traits, phenotypic expression, variability.

INTRODUCTION

Tomato is a valuable biological and informative organism that is widely used as a model for in vitro cultivation and various molecular biological, genetic engineering, and applied research (DOI 10.1038/nature11119; Saliba-Colombani *et al.*, 2000; Ohyama *et al.*, 2009; 2012; Lusser M. *et al.*, 2012; Gerszberg, *et al.*, 2015). Fundamental research contributes to significant advances in breeding and, conversely, breeders provide a wealth of information for tomato genetics. However, despite this, the effectiveness of the selection process is currently limited by the limited genetic variability of the cultivated tomato gene pool available for selection. The increasing deficit of genetic diversity and, as a result, the reduced adaptability of modern tomato varieties and hybrids to climate change require more active involvement of new sources of germplasm with more pronounced genetic divergence in the breeding process. Mutations are of particular interest in this regard. The presence of a large number of morphologically identifiable mutant genes allows us to address a wide

¹ Moldova State University, Institute of Genetics, Physiology and Plant Protection, 60, Alexei Mateevici str., MD 2009, Chisinau, Republic of Moldova.

range of issues in selection theory – localizing quantitative traits, studying the origin of cultivated species, identifying the characteristics of pleiotropic and non-allelic gene interactions, determining the level of recombination variability in F_2 – F_3 hybrid populations, and other phenomena (Zhuchenko, 1973; Kuzeminsky, 2004; Bocharnikova, 2011; Makovei 2022). The most important research on the influence of mutations on the formation of the cultivated tomato species (*Solanum lycopersicum* L.) was conducted by H. Stubbe (1966). The author was the first to experimentally prove the possibility of the emergence of cultivated tomatoes from wild forms as a result of gene mutations. Other researchers have come to the same conclusion about the progressive influence of mutant genes on tomato cultivation (Georgieva R., 1976; Avdeev, 2014). However, the problem of actively involving mutant tomato forms in breeding and genetic research remains unresolved to this day. The reason for this is the lack of research into the phenotypic expression of mutant genes in changing environmental conditions, as well as in different combinations within a single genotype. Of particular interest in this regard are marker genes that manifest themselves at the seed stage and control the synthesis of anthocyanin, cotyledon characteristics, and the first true leaves. Most of them do not have a pleiotropic effect and can be identified on the day of emergence (Kuzyminsky, 2004). This group is represented by a huge number of genes (Makovei, 2022), early identification of which in the germination phase will allow faster solving of breeding problems aimed at finding and identifying valuable genotypes with the desired combination of traits, as well as simultaneously analyzing and controlling the hybridity of splitting F_2 – F_3 populations. The active and effective use of the potential of mutant tomato forms in breeding and genetic research can be facilitated by their evaluation and classification into groups that differ qualitatively in terms of the specificity of manifestation and genetic variability of traits under different environmental conditions. It is also important to study the nature of the joint manifestation of several genes, since the same genes, acting separately, can differ fundamentally. This means that a comprehensive analysis and understanding of the nature of marker gene expression individually and in combination, including in relation to environmental conditions, will allow them to be used in a targeted manner to expand and enrich the genetic base of cultivated tomatoes with higher thresholds of economically valuable traits.

The aim of the research was to study the genetic potential of mutant tomato forms in terms of the nature of manifestation and degree of phenotypic expression of marker genes appearing at early stages of plant development – emergence of seedlings, cotyledon stage, and first true leaves.

MATERIALS AND METHODS

The experimental material consisted of 125 single- and multimarker mutant tomato forms from the collection of the Laboratory of Plant Genetic Resources,

Institute of Genetics, Physiology, and Plant Protection. These are mutations obtained using various mutagenic factors and spontaneous mutations that arose during the evolution of the crop, from genetic collections from different countries around the world (the USA, Germany, Bulgaria, Italy, and created in Moldova). The nature of manifestation and degree of phenotypic expression of marker genes in the early stages of ontogenesis – hypocotyl coloration, type and coloration of cotyledons and first true leaves – were determined according to gene nomenclature (Zhuchenko, 1973; Tanksley & Mutshler, 1989). The degree of phenotypic variability of marker traits depending on growing conditions (field, greenhouse) was determined using a 3-point system (3 points – stable expression of the trait, 2 – moderate variability, 1 – strong variability, or the trait is not expressed at all). The accounting and analysis of anthocyanin pigment on the hypocotyl of mutants and the variability of its index were determined using the coefficient of variation ($V < 10\%$ – stable manifestation, $V = 10\text{--}20\%$ – average variability, and $V > 20\%$ – strong variability) (Dospekhov, 1985). The description of the nature of morphological and biological characteristics and their variability during plant growth and development was carried out in accordance with the international descriptor (UPOV).

RESULTS AND DISCUSSION

Assessment and analysis of the nature of manifestation and degree of phenotypic expression of marker genes controlling anthocyanin synthesis, identified on the day of emergence and subsequent description of cotyledon and first true leaf markers, allowed us to establish the degree of genetic stability of each trait, individually for each genotype, both depending on the genes and their combination in a single genome, and on the conditions of plant cultivation. It has been shown that the collection contains a large number of genotypes, carriers of marker genes controlling anthocyanin synthesis (*a*, *aa*, *ag*, *al*, *atv*, *aw*, *bls*, *wv*, *etc.*). It has been noted that high temperatures (28°C and above) have a significant effect on the intensity of anthocyanin pigment, at which forms with *hp*, *ai*, *al*, and *atv* genes show a decrease in anthocyanin content, or the marker trait is not identified at all.

Taking into account the nature of phenotypic expression and the degree of variability of the trait, the collection samples were divided into corresponding groups (Table 1). Mutant forms (39 genotypes) were identified with a complete absence of pigment on the hypocotyl and stable expression of the trait during the vegetation period of plants under different growing conditions. Similarly, 12 genotypes had a stable expression of the trait, but with very strong anthocyanin coloration of the hypocotyl (from purple to black). 34 mutant forms were distinguished by the average degree of phenotypic expression of the trait characteristic of the tomato of the cultivated type. Weakly expressed, or barely

noticeable anthocyanin pigment on the hypocotyls had 40 mutants. In terms of the degree of phenotypic expression of the trait, significant differences were established both between single- and multi-marker mutant forms, as well as depending on the growing conditions of their plants (field, greenhouse), and within each of the groups (Table 1). A stable manifestation of the marker trait under different plant growing conditions was observed in single-marker forms, while the range of trait variability was wider in multi-marker mutants (Table 1). In individual analysis, the stable expression of anthocyanin pigment, or its complete absence, is observed in the following mutant forms: Mo 305(*aw*), Mo 343 (*aw*), 581 (*ag*), Mo 584 (*al*), Mo 588 (*aa*), Mo 651 (*al*), Mo 787 (*a*, *hl*), Mo 952 (*bls*). Similar results were obtained for a number of multi-marker mutants: – Mo 500 (*wo*, *d*, *aw*, *c*, *m-2*); Mo 504 (*aw*, *bk*, *d*, *o*, *p*, *s*, *Wo^m*); Mo 632 (*ag*, *h*, *t*, *u*, *pl*, *e*); Mo 638 (*V-2*, *c*, *a*, *u*, *ut*, *gs*, *gf*, *u*, *ms*); Mo 651 (*Xa-3*, *al*, *sp*); Mo 755 (*aa*, *wv*, *d*); Mo 779 (*ms-31*, *l*, *bu*, *dl*, *atv*); Mo 787 (*ms-2*, *a*, *hl*); Mo 851 (*clau*, *di*, *inc*, *ag*) and Mo 924 (*lg*, *vi*, *y*). The higher degree of variability in the phenotypic expression of the analyzed trait in multi-marker forms is due to different combinations of mutant genes in their genomes. For example, variability is strongly expressed in multi-marker mutants whose genomes combine sterility genes (*ps*, *s*, *ms*), potato leaf (*c*), and anthocyanin synthesis control genes (*a*, *aw*) – Mo 61, Mo 172, Mo 308, Mo 328, Mo 504, Mo 638, and others, which is of particular interest for their use in heterotic tomato breeding.

Table 1

Degree of phenotypic expression of marker genes controlling anthocyanin synthesis identified on the day of emergence and variability of trait indices depending on plant growing conditions

Genotype	Number of genotypes in the group	Coefficient of trait variability (V, %)		Mutant number in the collection		
		in field	in greenhouse			
Degree of trait expression						
Complete absence of anthocyanin						
Singlemarker mutants	18	1.6...6.3	5.7...9.8	Mo56, Mo396, Mo529, Mo562, Mo 576, Mo721, Mo61,	Mo311, Mo409, Mo556, Mo565, Mo588, Mo722, Mo172, Mo328,	Mo341, Mo442, Mo561, Mo566, Mo651, Mo835, Mo305,
Multimarker mutants	21	2.8...7.4	3.4...8.7	Mo308, Mo378, Mo584, Mo637, Mo759, Mo831,	Mo500, Mo620, Mo638, Mo779, Mo924,	Mo 343, Mo504, Mo632, Mo755, Mo787, Mo952

Table 1 (continued)

<i>The genotypes with strong anthocyanin pigment</i>				
Singlemarker mutants	5	2.3...9.8	7.4...11.6	Mo113, Mo163, Mo166, Mo536, Mo805
Multimarker mutants	7	4.4...13.5	6.7...12.5	Mo112, Mo451, Mo634, Mo781, Mo922, La1563, La2921
<i>Average degree of anthocyanin pigment expression</i>				
Singlemarker mutants	22	7.7...21.1	6.8...22.4	Mo122, Mo147, Mo316, Mo324, Mo382, Mo443, Mo446, Mo463, Mo466, Mo489, Mo534, Mo544, Mo555, Mo593, Mo594, Mo666, Mo724, Mo738, Mo900, Mo917, Mo918, La2802
Multimarker mutants	12	10.6...28.5	14.3...31.8	Mo74, Mo406, Mo516, Mo519, Mo600, Mo663, Mo718, Mo762, Mo791, Mo822, Mo851, La1159
<i>Weak anthocyanin pigment on the hypocotyl</i>				
Singlemarker mutants	29	2.3...18.1	9.7...15.4	Mo24, Mo35, Mo63, Mo120, Mo136, Mo304, Mo331, Mo339, Mo350, Mo377, Mo379, Mo392, Mo432, Mo460, Mo509, Mo518, Mo533, Mo558, Mo598, Mo603, Mo606, Mo670, Mo723, Mo732, Mo794, Mo833, Mo838, Mo917, La 2644, La2999
Multimarker mutants	11	7.6...29.6	8.4...41.8	Mo248, Mo334, Mo372, Mo385, Mo395, Mo508, Mo585, Mo640, Mo756, Mo762, Mo776,

Analysis of the phenotypic expression of this trait in single-marker and multi-marker mutants distributed into different groups showed that the widest range of variability is characteristic of genotypes with weak pigmentation. Depending on the combination of mutant genes, the trait index varied greatly, ranging from a complete absence of pigment to its significant enhancement, including depending on the conditions of plant cultivation. For example, as the air temperature increased, the anthocyanin pigment on different parts of the seedlings became barely noticeable, and at 30°C and above, it disappeared altogether. The coefficient characterizing the degree of trait variability in single-marker forms in field conditions varied from 2.3 to 18.1%, while in a greenhouse it varied from 9.7 to 15.4%. In multimarker mutants, it was higher and ranged from 7.6 to 29.6% in the

field and from 8.4 to 41.8% in the greenhouse (Table 1). Similarly high variability is characteristic of mutants with moderate pigment expression on the hypocotyl. Singlemarker forms from this group had a fairly high coefficient of variability, both when grown in the field and in a greenhouse, ranging from 7.7 to 21.1% and from 6.8 to 22.4%, respectively. In multimarker mutants, the variability in the phenotypic expression of anthocyanin pigment was even higher, with corresponding coefficients ranging from 10.6 to 28.5 in the field and from 14.4 to 31.8 in the greenhouse (Table 1). Such high heterogeneity in the nature of manifestation and degree of phenotypic expression of markers that control anthocyanin synthesis in mutant tomato forms at early stages of plant development under different growing conditions indicates the ambiguous action of genes controlling the trait and their interaction with others, carried by single-marker and multiple-marker mutant tomato forms.

Wide polymorphism was revealed within the collection, and in terms of the degree of distortion of the shape and color of cotyledonary and first true leaves, controlled by a large number of mutant marker genes: *aut*, *apn*, *alb*; *afl*, *div*, *ga*, *gil*, *c*, *cg*, *cla*, *clau*, *Cu*, *fla*, *ful*, *inf*, *inta*, *l*, *l-2*, *hur/+*; *lut*; *ltf*; *marm*, *Me*, *m-2*, *nv*, *op*, *res*, *rv*, *sy*, *syv*, *Tor*, *V-2*, *va-2*, *var*, *vo*, *V-5/+*, *wwd*, *wn*, *wv*, *wv-3*, *yg-2*, *Wo^m*, including various combinations of them in one genome. Based on the nature of the expression of color markers in cotyledon and first true leaves, the spectrum of their phenotypic expression is very wide – bright yellow, yellow, pale yellow, gray-green, light green, green, and dark green (Figure 1).

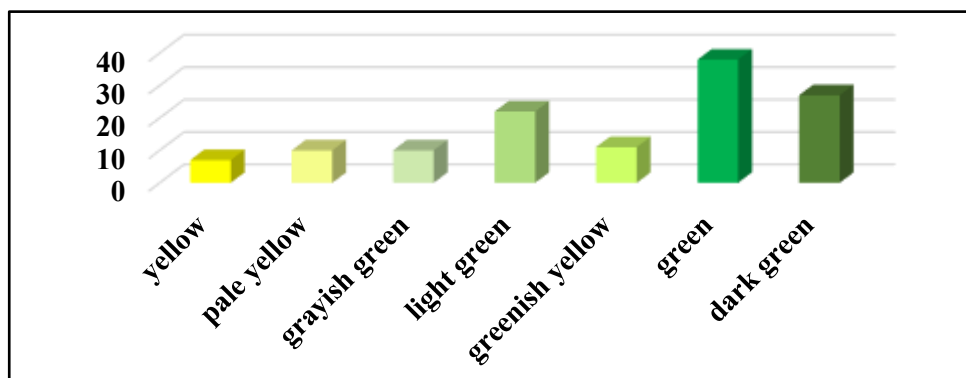


Figure 1. Differentiation and systematization of mutant tomato forms into groups based on the color of cotyledon and first true leaves.

Through individual analysis of the nature of these traits in each mutant at the cotyledon and first true leaf stages, the following genotypes were identified: – Mo 24; Mo 35; Mo 36; Mo 74; Mo 122; Mo 136; Mo 311; Mo 339; Mo 341; Mo 372; Mo 379; Mo 395; Mo406; Mo 409; Mo 500; Mo 508; Mo 529; Mo 536; Mo 547; Mo 566; Mo 585; Mo 589; Mo 606; Mo 632; Mo 634; Mo 638; Mo 640; Mo

663; Mo 666; Mo 755; Mo 756; Mo 759; Mo 762; Mo 776; Mo 781, which are carriers of the above genes. The information presented in Table 2 objectively reflects the characteristics of the phenotypic manifestation of marker genes that control the shape and color of cotyledonary and first true leaves, as well as their variability under different growing conditions. Single- and multi-marker mutants have been identified in which each marker trait controlled by the genes *afl*, *apn*, *atv*, *aut*, *c*, *cy*, *cla*, *clau*, *Cu*, *div*, *ga*, *inta*, *Ln*, *ltf*, *lur*, *lut*, *l-2*, *nv*, *Tor*, *Wom*, *yg-2*, is phenotypically clearly manifested and easily identified under different conditions ($V = 2.4...8.3\%$). In another group of mutant forms, there is a high degree of variability in the manifestation of marker traits controlled by the genes *alb*, *ful*, *l*, *m-2*, *marm*, *op*, *pg*, *res*, *rv*, *vo*, *wn*, *wv*, depending on their combination in a single genome and the conditions of plant cultivation. For example, the *alb*, *ful*, *m-2*, *op*, *pg*, and *marm* genes are clearly expressed in a greenhouse, but are weakly expressed or not identified at all in the field. Markers of mutant forms Mo 36 (*Va-2*), Mo 122 (*res*), Mo 663 (*vo*), Mo 666 (*wv*), Mo 755 (*wv*), are clearly expressed in the field, but are barely noticeable in the greenhouse (Table 2).

Table 2

Nature of marker trait expression at the cotyledon and first true leaf stages in tomatoes controlled by mutant genes under various plant growing conditions

Mutant number	Gene symbols and them names	The phenotypic expression of traits	Degree of phenotypic expression of a trait, score	
			in field	in green-house
<i>Singlemarker mutants</i>				
Mo 24	<i>wv</i> – <i>white virescent</i>	The cotyledons are pale yellow, and the first true leaves are light green. These characteristics are more pronounced in a greenhouse and barely noticeable in field conditions. They disappear completely in high temperatures and drought conditions.	1	3
Mo 35	<i>lut</i> – <i>lutea</i>	The cotyledons are yellow-green, the leaves of the seedlings are light green with dark veins, and they are equally distinct in the field and in the greenhouse.	3	3
Mo 36	<i>Va-2</i> – <i>varia-2</i>	The cotyledons are white-yellow, the tips of the first true leaves are light green, which during vegetation shift from the tips of the leaf lobes to their base.	3	2

Table 2 (continued)

Mo 74	<i>div – divaricata</i>	The cotyledons and first true leaves are light yellow. During the growing season, both in the field and in the greenhouse, the intensity of the colors changes and the leaves turn green.	3	3
Mo 122	<i>res – restricta</i>	The cotyledons are yellowish-light green and boat-shaped, and the lobes of the first leaves have purple veins. This marker trait is highly expressed in the field and is barely noticeable or disappears completely when plants are grown in a greenhouse.	3	1
Mo 136	<i>alb – albescent</i>	The cotyledons are grayish-yellow, the first true leaves have a high proportion of white variegation in the form of marbling, the spots are more pronounced at low temperatures. In the field, the spots disappear completely, in the greenhouse they are fixed until the end of the growing season.	1	3
Mo 311	<i>op – opaca</i>	The light coloration of the cotyledons and growth points of seedlings disappears completely as vegetation progresses.	2	1
Mo 339	<i>wv – white virescent</i>	Pale yellow cotyledons, chlorotic seedling growth point, white spots on leaves, which disappear completely in the field during intense heat and drought and persist longer in the greenhouse.	1	2
Mo 341	<i>Wo^m – Wooly</i>	Heavily pubescent hypocotyl, gray-blue pubescent cotyledons and all parts of seedlings and adult plants, including fruits.	3	3
Mo 379	<i>ful – fulgens</i>	The cotyledons are bright yellow, and the first leaves are light yellow. In the field, in intense heat, the leaves are golden yellow; in a greenhouse, this trait is not pronounced.	3	1
Mo 409	<i>nv – netted virescent</i>	Pale green cotyledons and first true leaves, which never turn green.	3	3
Mo 529	<i>Tor – Tortilis</i>	Seed leaves curled downward in the shape of a boat. The leaves of seedlings and mature plants retain this tendency under different growing conditions.	3	3

Table 2 (continued)

Mo 536	<i>cla</i> – <i>clara</i>	The yellow-green color of the cotyledons and first true leaves persists throughout the growing season, but the veins and petioles of the leaves are purple.	3	3
Mo 547	<i>gil</i> – <i>gilva</i>	The cotyledons are intensely yellow. The growth points of the seedlings are bright yellow, lethal.	1	1
Mo 566	<i>yg-2</i> – <i>yellow green-2</i>	Yellow-green cotyledons and first true leaves. Seedling and adult plant growth points are light yellow. Marker is more pronounced in the field.	3	1
Mo 589	<i>apn</i> – <i>albopunctata</i>	Uniform white spotting on cotyledons and first true leaves, which disappears as the plant grows and the leaves turn green.	3	3
Mo 606	<i>Cu</i> – <i>Curl</i>	The cotyledonary and first leaves are light green, with greatly shortened midribs and petioles, resulting in significant leaf curling.	3	3
Mo 794	<i>afl</i> – <i>albifolium</i>	The cotyledons and leaves of seedlings are gray-green, becoming green in mature plants.	3	3
Multimarker mutant forms				
Mo 378	<i>l</i> – <i>lutescent</i> <i>c</i> – <i>potato leaf</i> <i>dd</i> – <i>double dwarf</i> <i>aa</i> – <i>anthocyanin absent</i>	Seed leaves and first leaves are gray-green. Adult plants show premature yellowing and leaf fall, which is more pronounced in field conditions. In a greenhouse, the leaves are light green.	3	1
Mo 395	<i>rv</i> – <i>reticulate virescent</i> <i>og</i> – <i>old gold</i>	Seed leaves and seedling leaves are gray-green with dark veins; as vegetation progresses, the marker trait (<i>rv</i>) is not identified.	1	1
Mo 406	<i>ga</i> – <i>galbina</i> <i>dl</i> – <i>dialytic</i> <i>wd</i> – <i>wilty dwarf</i>	Pale green cotyledons and first true leaves. Leaves of adult plants with a grayish coating in the field and in the greenhouse.	3	3
Mo 500	<i>Wo^m</i> – <i>Wooly</i> <i>d</i> – <i>dwarf</i> <i>aw</i> – <i>without anthocyanin</i> <i>o</i> – <i>ovate</i> <i>r</i> – <i>yellow flesh</i> <i>m-2</i> – <i>mottled-2</i> <i>c</i> – <i>potato leaf</i>	The cotyledons are light green and heavily pubescent (<i>Wo^m</i>), and the leaves of the seedlings are dotted with chlorotic spots (<i>m-2</i>), which disappear during vegetation in field conditions but remain in the greenhouse.	2	3

Table 2 (continued)

Mo 508	<i>sf</i> – <i>solanifolia</i> <i>sy</i> – <i>sunny</i> <i>alb</i> – <i>albescent</i> <i>mua</i> – <i>multifurcata</i>	Seed leaves are pale yellow to pale green, the first true leaves are entire, pale yellow, later becoming uniformly green in the field and in the greenhouse, but curling into a tube.	3	3
Mo 632	<i>ag</i> – <i>anthocyanin gainer</i> <i>h</i> – <i>hairs absent</i> <i>l-2</i> – <i>lutescent</i> <i>u</i> – <i>unif. ripening</i> <i>pl</i> – <i>perlucida</i> <i>lg</i> – <i>light green</i>	Green coloration of cotyledonary and first true leaves, which become light green and then light yellow as seedlings and adult plants grow. Marker trait (<i>l-2</i>) is strongly expressed under various plant growing conditions.	3	3
Mo 634	<i>per</i> – <i>perviridis</i> <i>c</i> – <i>potato leaf</i> <i>r</i> – <i>yellow flesh</i> <i>l</i> – <i>lutescent</i> <i>alb</i> – <i>albescent</i> <i>fla</i> – <i>flavescent</i>	The cotyledons are green, and the first leaves are dark green with strong variegation in the form of marbling. At low temperatures, the trait (<i>alb</i>) is clearly visible, while at high temperatures it disappears. It is practically invisible in the field, as the plants wilt and wither early.	1	3
Mo 638	<i>v-2</i> – <i>virescent-2</i> <i>c</i> – <i>potatoleaf</i> <i>a</i> – <i>anthocyanless</i> <i>y</i> – <i>colorless fruit epidermis</i> <i>clau</i> – <i>clausa</i> <i>gs</i> – <i>green stripe</i> <i>gf</i> – <i>gametophytic factor</i> <i>r</i> – <i>yellow flesh</i> <i>mc</i> – <i>macrocalyx</i>	Complete absence of anthocyanin on the hypocotyl, cotyledons, stem, leaves of seedlings and adult plants. Gray-yellow cotyledons, first true leaves are pale green with faint purple veins. Leaves of adult plants are intensely green. All marker traits are phenotypically equally expressed in field and greenhouse conditions.	3	3
Mo 640	<i>inta</i> – <i>integrifolia</i> <i>yg-4</i> – <i>yellow-green-4</i>	The leaves are chlorotic, whitish-yellow-green. In seedlings and adult plants, the leaves are yellow, short, with serrated and twisted edges, clearly visible under different growing conditions.	3	3
Mo 663	<i>rvt</i> – <i>red vascular tissue</i> <i>vo</i> – <i>virescent</i> <i>orange</i> <i>d</i> – <i>dwarf</i> <i>gf</i> – <i>green flesh</i> <i>sp</i> – <i>self pruning</i>	The cotyledons are yellow-green, and the first true leaves are pale green. The leaves of mature plants range from pale green to gray-green. In a greenhouse, they become intensely green.	3	2

Table 2 (continued)

Mo 666	<i>Me</i> – Mouse ears <i>wv</i> – white virescent	The cotyledons are pale yellow. The first true leaves are chlorotic with white spots, which disappear in the greenhouse. The growing point of the plants is light yellow, a trait that is more pronounced in the field.	3	2
Mo 755	<i>aa</i> – anthocyanin absent <i>wv</i> – white virescent <i>d</i> – dwarf	Complete absence of anthocyanin in all parts of the plant. Seed leaves are pale yellow; seedling leaves are white-spotted and chlorotic. In the greenhouse, as vegetation progresses, the spotting disappears and the leaves turn green.	3	2
Mo 756	<i>ru</i> – rutilis <i>st</i> – sterile <i>sy</i> – sunny	Seed leaves are narrow, pale green-gray to yellow, true leaves are light green. Plant growth point is pale yellow to intense yellow.	3	3
Mo 759	<i>bls</i> – baby lea syndrome <i>aut</i> – aureata	The cotyledons are yellow. The golden-yellow growth points and leaves of young seedlings; as they grow, this marker disappears and is practically undetectable in adult plants.	3	3
Mo 762	<i>ful</i> – fulgens <i>e</i> – entire <i>ra</i> – rava	Yellow-green cotyledons and light green in seedlings. In adult plants, the leaves are green when grown in a greenhouse and yellow in the field.	3	1
Mo 776	<i>var</i> – variabilis <i>not</i> – notabilis	Seedling leaves and first true leaves are light green in color. The growing point of seedlings and adult plants is yellow-green. This trait is clearly visible in the field and less pronounced in a greenhouse.	3	2
Mo 781	<i>wd</i> – wilty dwarf <i>marm</i> – marmorata	The cotyledons and leaves of seedlings are gray-green. The first true leaves and subsequent leaves are covered with marbled blurred spots of various shades ranging from white to light green. The marker is clearly expressed in the greenhouse and does not appear in the field.	1	3

The remaining marker genes from this group can only be identified at a specific stage of plant growth and development (Table 2). Overall, the variability in the nature of manifestation and degree of phenotypic expression of marker traits in this group of genotypes was very high, with a corresponding coefficient ranging

from 21.6 to 97.4%. A number of mutants (*div*, *icn*, *ven* and others) are difficult to identify both in the field and in the greenhouse. At the same time, it should be noted that the *gill*, *Xa*, *Xan*⁺, and *Xan-3* genes are only expressed at the cotyledon stage, which is intensely yellow, and the homozygote is nonviable. Among those studied, there are also mutants carrying the marker genes *a*, *aw*, *bs*, and *e*, which are identified at the seed stage (due to the phenotypic expression of different alleles) and can be effectively used in heterozygous seed production.

Such a variety of mutant tomato forms in terms of the manifestation of marker traits controlled by a huge number of easily identifiable genes in the early stages of plant development, including depending on the conditions of plant cultivation, opens up wide opportunities for their active involvement in breeding and genetic research. Based on the results obtained, it is possible to study the correlations between markers that appear at the seedling stage and other economically valuable traits that are only found in adult plants. This will allow us to work with a large number of genotypes, including extensive splitting populations obtained from different hybrid combinations, identifying valuable genotypes with a high degree of correlation between traits at different stages of plant growth and development, thereby reducing the time required for research aimed at creating new tomato varieties with an original combination of mutant markers and a range of other economically valuable traits.

CONCLUSION

Collectible mutant forms (125 genotypes) have been identified and classified into corresponding groups based on phenotypic expression and degree of variability in anthocyanin coloration on the hypocotyl, by type, shape, and color of cotyledon and first true leaves, controlled by a large number of mutant genes that can be easily identified on the day of emergence in single- and multimarker forms when growing their plants under different conditions (field, greenhouse).

A more stable character in terms of the degree of phenotypic expression of marker traits under different plant growing conditions is observed in singlemarker mutant forms, while the variability of traits is significantly higher in multimarker mutants.

Single- and multimarker mutants have been identified in which each marker trait controlled by the genes *afl*, *apn*, *atv*, *aut*, *c*, *cy*, *cla*, *clau*, *Cu*, *div*, *ga*, *inta*, *Ln*, *ltf*, *lur*, *lut*, *l-2*, *nv*, *Tor*, *Wo*^m, *yg-2*, is phenotypically clearly manifested and easily identified under different plant growing conditions ($V = 2.4...8.3\%$), which are included in breeding programs to improve existing tomato varieties and obtain new ones.

BIBLIOGRAPHY

1. Avdeev A.Yu., 2014, Spontaneous mutations in tomatoes in terms of size, shape, fruit weight, and trait evolution. *Reports Russian Academy of Agricultural Sciences*. No. 1. pp. 16–19.
2. Bocharnicova N.I., 2011, Genetic collection of tomato mutant forms and its use in the breeding and genetic researches. Moscow, 120 p., ISBN 978-5-901695-46-3 (In Russ).
3. Georgieva R., 1976, Genus *Lycopersicon* Mill. Biosystematics and genetic research. Sofia: Bulgarian Academy of Sciences. Publishing House, 564 p.
4. Gerszberg, A., Hnatuszko-Konka A. K., Kowalczyk T., Kononowicz A.K., 2015, Tomato (*Solanum lycopersicum* L.) in the service of biotechnology. *Plant Cell, Tissue and Organ Culture* (PCTOC), Vol. 120. pp. 881–902.
5. Dospehov, B.A., 1985, Experimental methodology. M.: Agropromizdat, 416 p. ISBN: 9785-458-23540-2. (In Russ).
6. Zhuchenko, A.A., 1973, Genetics of Tomatoes. Kishinev. 664 pp. (In Russ)
7. Kuzyomensky A.V., 2004, Breeding and Genetic Studies of Mutant Forms of Tomato. Kharkov, ISBN 966-8431-00-6 (in Russ).
8. Lusser M., Parisi C., Plan D., Rodriguez-Cerezo, E., 2012, Deployment of new biotechnologies in plant breeding. *Nature biotechnology*. No 30. pp. 231–239.
9. Makovei M.D., 2022, The potential of mutant forms of tomato for selection and genetic studies. Sprint “Print-Caro”, Chisinau., 208 p. ISBN 978-9975-165-22-8 (In Russ).
10. Ohya A., Asamizu E., Negoro S., Miyatake K., Yamaguchi H., Tabata S., Fukuoka H., 2009, Characterization of tomato SSR markers developed using BAC-end and cDNA sequences from genome databases. *Molecular Breeding*, 23(4):685-691. DOI 10.1007/s11032-009-9265-z.
11. Saliba-Colombani V., Causse M., Gervais L., Philouze J., 2000, Efficiency of RFLP, RAPD, and AFLP markers for the construction of an intraspecific map of the tomato genome. *Genome*. 43:29–40. DOI 10.1139/g99-096.
12. Stubbe G., 1966, On the connections between natural and artificially obtained diversity of mutant forms and on some experimental studies on the evolution of cultivated plants. *Genetics*, No. 11. pp. 9–30.
13. Stubbe H., 1971, Weitere evolutionen genetische Untersuchungen in der Gattung *Lycopersicon*. *Biologisches Zentralblatt*, No 90. Bd. 5. ss. 545–559.
14. Tanksley S.D., Mutschler M.A., 1989, Linkage map of the tomato (*Lycopersicon esculentum*). *Genetic map.*, pp. 6.3–6.15.
15. Tomato - UPOV (*Solanum lycopersicum* L.) V 2012 0007 TG/44/11 Rev. Geneva.

ACKNOWLEDGMENTS

The research was carried out in the Project in the field of science and innovation 25.80012.5107.15SE, „Diversitatea genetică a genelor marker mutante de tomate (*Solanum lycopersicum* L.) ca sursă de germoplasmă nouă pentru crearea de soiuri și hibrizi heterotici capabili să realizeze potențialul de productivitate în condițiile schimbărilor climatice”, financed by the Ministry of Education and Research.

