

GENETICA, BIOLOGIA MOLECULARĂ ȘI AMELIORAREA

TRANSGENERATIONAL EFFECTS OF VIRAL INFECTION ON QUANTITATIVE TRAITS MANIFESTATION IN TOMATO OFFSPRING DERIVED FROM INFECTED PLANTS

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Rezumat

S-au stabilit diferențe statistice pentru valorile medii ale caracterelor cantitative în generația a patra (G_4) descendentă din plantele infectate cu VAT sau VMT comparativ cu controlul a 5 genotipuri de tomate cu reactivitate diferită la infecția virală. Rata caracterelor cu diferențe depinde de combinația virus-genotip. VAT a contribuit la manifestarea unei rate mai mari a caracterelor cu abateri de la control comparativ cu VMT. Reinfecția plantelor în generația G_3 cu VAT sau VMT, a condus la manifestarea unei rate relativ mai mici de

caractere cu devieri în G_4R comparativ cu variantele G_4 care nu sunt supuse reinfectării.

Cuvinte cheie: VAT – virusul aspermiei tomatelor, VMT – virusul mozaicului tutunului, infecții virale, caractere cantitative, efecte transgeneraționale

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Introduction

The phenotypic changes produced by pathogens in compatible hosts are more easily detectable and appropriately better known for plant-pathogen systems. The presence of pathogens in the plant leads to changes in metabolic processes, and also affects the functionality and structure of the genetic material. In this context, the consequences of host-pathogen interactions on the offspring of affected plants are of great interest. Some researches on limited plant-virus systems (*Nicotiana tabacum*, *Arabidopsis thaliana* and *Brassica napus*), indicate the manifestation of genetic and epigenetic effects in the offspring of affected hosts [3, 6], and the memory of stress conditions through genetic-epigenetic modifications can be maintained for several generations. Architectural changes in plants (including cellular components, molecular functions and biological processes) as result of in post viral infection action are specific depending on the type of interaction (resistant, sensitive) and aim to facilitate the response of the plant in case of repeated stress in their offspring [5]. According to these authors, the studies clearly demonstrate that pathogens trigger a limited transgenerational effect; however, to explore transgenerational diversity and specificity, more types of plant-pathogen groups need to be used as stressors in the parent generation. The inheritance of epigenetic changes induced by environmental factors could be adaptive, but could also be harmful, given that the parent's epigenetic environmental memory can affect the responses in offspring [4]. In the context of the above, the study presented aims to attest to the particularities of phenotypic changes in the offspring of TAV or TMV infected plants, as well as the effect of repeated infection on quantitative traits in tomatoes depending on the status of the genotype –virus interaction (compatible, incompatible).

Material and methods

Five tomato genotypes were studied: sensitive variety Elvira, spontaneous form *Solanum pimpinellifolium*, genotypes with TMV-resistance genes – Craigella TM1 (Tm-1/Tm-1), Craigella TM2 (Tm-2²/Tm-2²), Rufina (Tm-1/Tm-2²). At the stage of four leaves plants were inoculated with TAV (tomato aspermy virus, tripartite positive-sense single stranded RNA, Cucumovirus), TMV (tobacco mosaic virus, single linear positive-sense single strand RNA, Tobamovirus) or mock inoculated. Manifestation of traits were evaluated in the fourth generation (G_4). Two variants for G_4 generation were analyzed: i) G_4 healthy descendants from G_0 TAV or TMV infected plants; ii) G_4R – healthy descendants from G_3 reinfected with TAV or TMV. For each type of viral infection were analyzed 3 variants: i) control – healthy plants, uninfected, ii) G_4 , iii) G_4R . All plants were grown under similar conditions. In order to eradicate pathogens and avoid transmitting the infection to G_4 plants, the seeds were heat treated

for 72 hours at 70° C. The analyzed plants did not show symptoms specific to TAV or TMV infection. For the genotypes with determined growth (Elvira and TM1). 22 biomorphological traits were analyzed, and for those with indeterminate growth (*S. pimpinellifolium*, TM2, Rufina) - 25 traits.

Data processing was performed using the Statgraphics Plus 5 statistical software package and Excel applications. The dispersion analysis was performed based on the Anova test, the distribution of quantitative traits in clusters, based on the cluster analysis.

Results and discussion

The presence of viral infections in plants is manifested in the host by considerable changes in all metabolic processes, reflected in the quantitative traits too. In some works, the effects of the infection on the plant offspring, considering causal aspect at the level of methylation and recombination [3], metabolic processes [8] or indicating inherited resistance and seed architecture modification were attested and the response is differentiated from sensitive to resistant genotypes [5].

The evaluation of the quantitative traits in the case of the genotypes with different reactivity to TAV or TMV established that over several generations there are significant deviations according to the distribution spectrum of values, as well as, the average values of traits compared to control plants, uninfected by viruses. Comparative analysis of the same genotypes shows a decrease in the rate of traits with significant deviations from the control in G₄ descendants from plants infected with TAV or TMV compared to the G₃. Depending on the genotype and the applied infection, the rate of traits with significant deviations from the control was different. Rufina and Elvira genotypes exhibited the lowest number of traits with significant deviations from the control for TAV and TMV variants, the rate of traits being 16 and 20% and 14 and 27 %, respectively (Fig.1). The genotypes TM1 and TM2, by the way as well as Elvira, showed statistically confirmed deviation of traits with obvious differences between rate in case of TAV and TMV. For example, in case for the genotypes Elvira, TM2 and *S. pimpinellifolium*, in the populations obtained from TAV-infected plants a relatively higher quota of traits with significant deviations was established (by 27, 32 and 40%, respectively), compared to the descendants from TMV (by 14, 16 and 32%). The highest rate of traits with differences of 45 % was attested for TM1/TMV variant.

The G₄R variants descendant from G₃ TAV or TMV reinfected plants reveal two different tendency for the number of traits with deviations from the control compared to the G₄ variant: 1. Relatively smaller rate of traits with deviations compared to the same non-reinfected ones, was attested for the genotypes TM1, TM2 and Elvira (e.g. rate of traits with deviation for TM1/TAV consists in G₄ -32% and G₄R -18%; and TM1/TMV in G₄ - 45 % and G₄R - 23%). 2. Rate was relatively higher or similar in reinfected G₄R variants compared to G₄ one, established for Rufina and *S. pimpinellifolium* genotypes (Fig.1.).

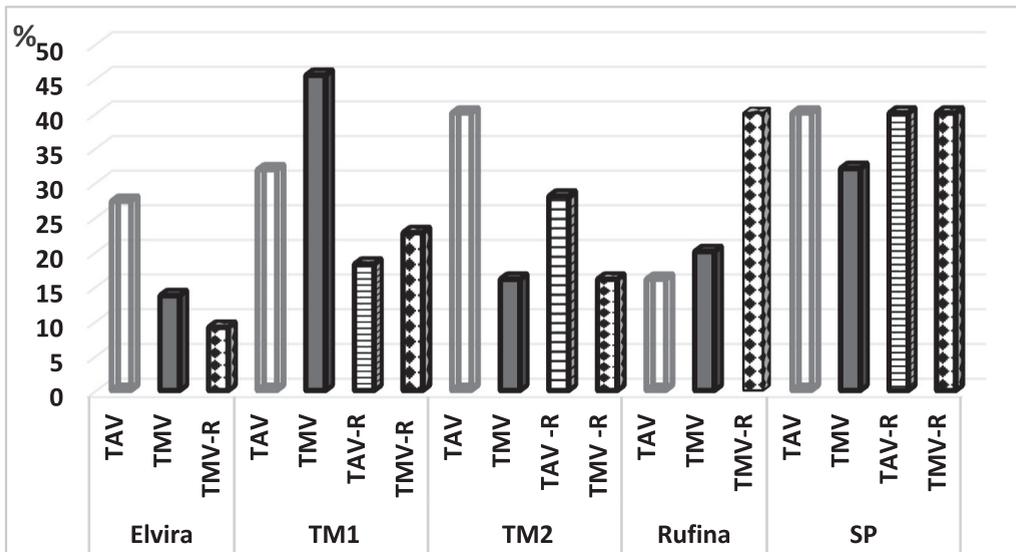


Figure. 1. The rate of traits with significant differences from control for descendants G_4 and G_4R (reinfected) with TAV and TMV, %. TAV-R and TMV-R variants reinfected in G_3 .

Statistical comparison of the average values of the traits in the G_4 populations (descendent from infected variants in G_0) with G_4R (reinfected in G_3) in almost of cases established similar quotas of traits with deviations comparison with the control.

In the case of traits with statistically significant differences from the control, 2 events were possible: (i) The average values of the traits were higher than in the control, and therefore in appearance the more efficient G_4 generation; (ii) Mean values indicated lower values, thus attesting to genotype downgrading.

In this aspect, the most vulnerability proved the Elvira variety for which 83 and 100% of traits were with significant deviations indicating lower values than the control in G_4 , for the TAV and TMV variants, respectively. Also, in this category is the G_4 variant descended from plants infected with TAV of the Rufina genotype, for which out of 16% of traits with significant deviations from the control, 100% indicated lower values than the control.

However, for some genotypes was showed a relatively large number of traits that indicated higher values than the control. Such, for the TM2 genotype were noted 80 and 100% for TAV and TMV variants respectively, the *S. pimpinellifolium* – a spectrum of 60 and 88% of traits, and the TMV variants for Rufina genotype with 60% and for TM1 with 70% of the traits with higher values than the control (Fig.2). Thus, it was found that the rate of traits with higher values than the control for genotypes TM1, TM2, and Rufina in the case of incompatible interaction (TMV) was higher compared to the compatible one (TAV).

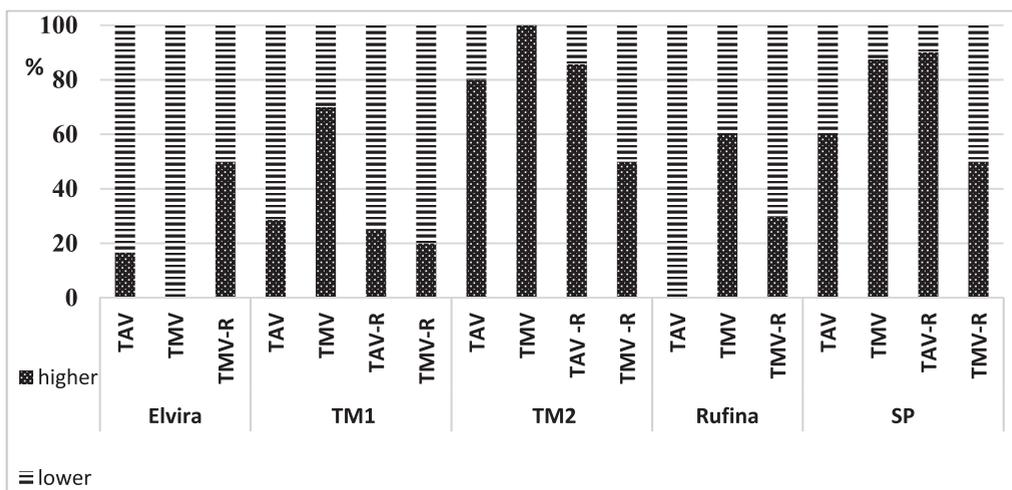


Figure. 2. Traits rate with statistical higher or lower average values than the control, %.

In most of cases, G_4 descendants reinfected in G_3 (TAV or TMV) expressed lower values (for 50-80% of traits) for the some traits (with significant deviations) compared to the control. Only the genotypes TM2 and *S. pimpinellifolium*, reinfected with TAV indicated a rate of 86 - 90% traits with higher values than the control.

Thus, although the quota with differences from the control is relatively small in reinfected plants, these traits more frequently indicate lower values than the control, while in the case of uninfected G_4 offspring, the most variants marked higher values than the control (more than 60% of traits with statistically significant differences). Only certain virus-genotype combinations showed a rate of more than 60% of traits with lower values than the control (Elvira / TAV and TMV, TM1 / TAV and Rufina / TAV) (Fig.2).

Among the variants that indicated traits with statistically different values, more performing to the control are enumerated:

- TMV variant of the Rufina genotype: a great number of clusters on the main axis with shorter distance between I-IV clusters, accompanied by an increase of average value of the fruit weight.
- The TMV variant of the TM1 genotype: an increase number of total fruits per plant by 56.3% more than in the control, and increased productivity by 30.5%;
- The TAV and TMV variants of the TM2 genotype: considerable increase of the plant height, number of shoots on main axis and the distance between I-IV clusters.

For various TAV or TMV variants of the studied genotypes, was attested that some traits with deviation from the control in consecutive generations G_3 and G_4 manifest a stability. Thus, the distance between the clusters I-IV and the number of internodes between them expressed differences for the TAV and TMV variants in TM2, *S. pimpinellifolium* and Rufina genotypes. In the case of TM1, both TAV and TMV, led to significant differences, by increasing the values of the number of flowers and fruits per cluster II; TMV in particular contributed to the significant increase in the number of fruits per cluster III, the total number of clusters per axis and the total number of fruits per plant (by 2.1 times compared to the control).

The clustering analysis revealed some peculiarities of the distribution of traits in clusters depending on the previous phytosanitary status of the plants. For the TM1

genotype, statistical differences between variants according to the number of fruits per plant were established. By the way, a different neighborhood, as well as the distance between the number of fruits per plant and other traits in descendants of plants infected with TAV or TMV compared to the control have been attested (Fig. 3).

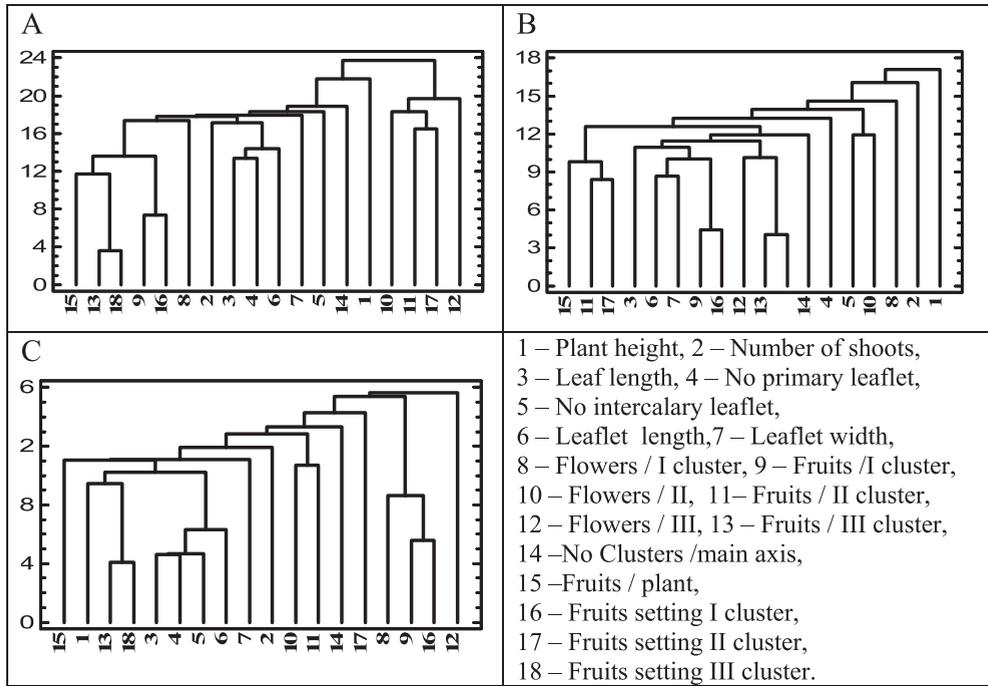


Figure 3. Clustering distribution of quantitative traits in G₄ of TM1 genotype, according to the previous phytosanitary status: A – Control, B –TAV and C – TMV variants (Nearest Neighbor Method, Distance – Squared Euclidean).

For example, in the TMV variant, the "Number of fruits per plant" is in a supercluster with the "Plant height" and "Leaf length", the relations not attested in the control, but also have in common "Number of fruits" and "Fruit setting coefficient on III cluster". Also, the configuration of clusters in TAV variant is different from the control and the TMV variant. The analysis of the manifestation of 4 quantitative traits ("Plant height", "Number of shoots per main axis", "Leaf length between I-II clusters", "Number of clusters per main axis") established that the descendants of plants infected with TAV or TMV show significant differences compared to the control for 4 of 5 analyzed genotypes (except Elvira) for at least one trait. At the same time, the traits which marked differences from the control for more variants with TAV or TMV are: "Number of shoots per main axis", "Leaf length" or "Number of clusters per axis", proving a major plasticity. At the same time there were variants (TM1 / TMV, TM2 / TAV and TM2 / TMV) that expressed differences for the idem traits combinations.

In the context of these, the depressional analysis established a significant variation of plant morphological parameters, according to the genotype and trait, as well as of the interaction *genotype x virus*, *genotype x trait* and *genotype x virus x trait* factors (Table).

Table. Analysis of variance for some morphological traits

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Main effects					
A: Genotype	23852.6	4	5963.16	134.87	0.0000
B: Virus	23.3814	2	11.6907	0.26	0.7677
C: Traits	428278.0	3	42759.0	3228.76	0.0000
Interactions					
AB	1481.68	8	185.21	4.19	0.0001
AC	55109.8	12	4592.49	103.87	0.0000
BC	254.518	6	42.42	0.96	0.4519
ABC	2623.68	24	109.32	2.47	0.0001
Total	547853.0	755			
A: Plant height					
AB	3853.09	8	481.4	2.99	0.0037
A: Number of shoots					
AB	74.80	8	9.35	4.65	0.0000
A: Leaf length					
AB	157.30	8	19.66	1.52	0.1536
A: Number of cluster per axis					
AB	20.17	8	2.51	4.37	0.0001

The contribution of the *genotype* and *virus* factors on the variability of the traits established a significant impact for "Plant height", "Number of shoots" and "Number of clusters per axis". The variance of these traits was influenced by viral infection, the effect of which had a statistical impact dependent on genotype.

Unfortunately, we could not find any published literature regarding the study of inter- or transgenerative effects on phenotypic changes in tomato in case of infection or reinfection with TAV or TMV for compatible-incompatible interaction that could confirm our findings.

Taking into account the response mechanisms to biotic and abiotic factors, we can assume that transgenerationally produced changes in the plant as a measure of adaptation under the adaptively action to stress factors may be advantageous in plant improvement [9, 2] or may have a negative impact, preventing the plant in full potential manifesting. In intergenerational aspect, in the case of repeated factor action, the plants react prompt and adequate, assuming low consumption of total metabolites [1, 7].

Summarizing the obtained results we can conclude, the modification of quantitative traits expression in tomato offspring of plants infected with viruses (TAV or TMV), as well as the specific change in the degree of manifestation under reinfection with differential rate in susceptible and resistant genotypes, which reflects adaptability to extensive factors.

Conclusions

1. In G_4 descendants of virus infected plants of 5 tomato genotypes with different types of response to viral infection (sensitive, tolerant, resistant), there were significant differences from control according to the average values of a wide range of architectural and productivity traits.

2. The rate of traits with statistic differences depends on the virus-genotype combination. For most of the genotypes (except TM1), TAV contributed to the

manifestation of a higher rate of traits with deviations from the control comparatively to TMV. In the same time, TMV determined the manifestation for smaller average values of traits in case of significant differences for Elvira and, in contrary, 100% of the traits indicated higher values for TM2. In the same way, TAV induced lower average values for all traits with significant differences for Rufina genotype.

3. Reinfection of plants in the third generation G_3 with TAV or TMV, led to the manifestation of a relatively lower rate of traits with deviations in G_4R compared to G_4 variants not subjected to reinfection, but these traits usually indicate statistically lower values than the control. However, TM2 and *S. pimpinellifolium* genotypes, reinfected with TAV indicated a rate of 86 - 90% traits with higher values than the control.

4. The dispersion analysis established a significant contribution in the variation of the traits by the *genotype* as well as by the interaction of the factors: *Genotype x virus*, *Genotype x Traits* and *Genotype x Virus x Traits*.

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