THE RESPONSE OF OFFSPRING OF VIRUS-INFECTED TOMATO PLANTS TO ABIOTIC FACTORS AT THE GAMETOPHYTIC AND SPOROPHYTIC LEVELS

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Abstract

The investigation assesses the influence of abiotic factors (heat/drought) on some biomorphological traits of sporophyte and male gametophyte in the virus-free offspring from virus-infected tomato plants (Tobacco Mosaic Virus or Tomato Aspermy Virus). The variance analysis of the evaluated traits, in both sporophyte and gametophyte, under abiotic stress conditions revealed, as a rule, a significant contribution of stress in the variability, followed by the effects of genotype and plant health status with different strength. The analysis confirmed the significant influence of genotype (5.6...19.7%), heat (21.7...81.5%), drought (55.4...82.1%), health status (2.9…36.8%), and their interactions on the variability of male gametophyte traits. In most cases, the influence of stresses caused suppression of the evaluated traits' values. Under conditions of heat or drought, depending on plant' health status, it was confirmed the specific manifestation of some traits in sprout (radicle length, growth intensity), plant (plant height, number and leaves length) and male gametophyte (pollen viability, pollen tube length). Thus, specific effects expressed by stimulation, inhibition or lack of differences for the analysed traits were observed in the offspring of virus-infected plants under heat or drought conditions compared to the optimal one. Analysis of pollen variability spectra for each genotype showed differences in sensitivity to the action of the factors, which allows description of the microgamete reaction to stress and application of the obtained data for predicting sporophyte resistance.

Key words: Abiotic stress, gametophyte, plant health status, sporophyte, virus

The action of biotic and abiotic stress factors, as well as their combination, contributes to a considerable decrease in yield in crops. In this context, the identification of resistant genotypes to the associated action of unfavorable environmental factors is of great interest (Oshunsanya S. et al., 2019). However, in traditional practice, the creation of genotypes resistant to associated stresses is quite difficult, because of specific peculiarities invoking independent reaction and selection for each type of resistance. Solving this task involves increasing plant adaptability by using methods of genotypes' reactions analys at haploid/diploid level under stressful conditions with subsequent selection of perspective forms.

Seed germination is considered to be one of the most sensitive plant stages which is strongly influenced by various environmental stressors including temperature and water deficit (Foolad M. et al., 2007). Research encompassing morphological indicators of tomato sprouts under optimal and heat or drought stress conditions is important and relevant, stemming from the existence of genotypic and phenotypic correlation of resistance at the sprout and plant stage, as well as in the sense of the consequences of stress at early developmental stages on the later ones (Kazmi R. et al., 2012). Associative observations have established that viral infections could under certain circumstances improve plant tolerance to abiotic stress, involving various regulatory mechanisms - osmoprotective and antioxidant (Xu P. et al., 2008), efficienct using of water in plant (Pagliarani C. et al., 2022), by the way involving differential responses in susceptible and resistant genotypes to pathogens (Anfoka G. et al., 2016). In various studies transgenerational effects in the offspring of virus-infected plants, expressing significant changes in the metabolic profile, as well as in resistance to various factors has been attested (Bilichak A., Kovalchuk I., 2016; Luna E. et al., 2011).

Under abiotic stresses conditions in a large number of species it has been established decreased pollen performance both *in vitro* and *in vivo* (Prasch C., Sonnewald U., 2013; Razaq K. et al., 2017). It was found that the influence of abiotic stresses during the development of the male reproductive organs correlates with decreased fruit set in tomato (Sato S. et al., 2000). At the same

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time, there is information on the specific reactions of pollen grains to the stressogenic action, which allows the identification of genotypes resistant to this stage (Boavida L., McCormick S, 2007; Firon R. et al., 2006). It has been found that pollen viability is an important tolerance trait of genotypes to abiotic stresses (Johnson M. et al., 2006). Research results (Razaq K. et al., 2017) showed that the change in *Helianthus annuus* L. pollen viability under heat stress was largely controlled by genetic variability, which indicates that the breeding efficacy by this trait can be improved by recurrent selection. To improve male gametophytic quality and increase the adaptability of the reproductive system to the impact of abiotic and biotic factors, valuable importance belongs to the range and type of genetic variation, associated with pollen viability (Kalyar T. et al., 2014).

In this context, it is of interest to elucidate the contribution of genetic and environmental (high temperature/hydric stress) factors in the variation of male gametophyte and sporophyte functional characters in the progeny of virus-infected plants.

MATERIAL AND METHOD

The following genotypes were included in the research: *S. pimpinellifolium, S. chilense*, Mary Gratefully, Jacota, Flacara, Tomis, Venet, Rufina and Mihaela. All genotypes were evaluated in three different health status: progeny from plants infected with Tobacco mosaic virus (TMV), Tomato aspermy virus (TAV) and healthy – Control (C). In order to inactivate the pathogens, the seeds from virus infected plants as well as control ones were previously exposed to heat treatment at 70^{0}C for 72 hours.

Sprouts. Heat and drought stress at the germination stage was modelled in Petri dishes. After 72 hours of germination (25^{0}C), the heat stress (42^{0}C for 6 h) or water deficit (6.5% sucrose solution, 4.65 atm., 25^{0}C) was applied. For each genotype 2 repetitions each of 50 seeds in control and treatment variants were included. The roots length were measured until and after the stress application (72 h poststress), and the obtained values were used to determine the intensity of root growth (K) – as ratio of final to initial length. Resistance was calculated according to the formula: R = K1/K2 x 100 (%), where K1 – represent the ratio of the final value of the root length to the initial length in the heat stressed variant, K2 – similarly, the ratio of root length in the control variant. For drought variants K1 and K2 represent differences between the final values of the root length to the initial length.

Plants. Plants about 5 weeks old, progeny form healthy (control - C) and TMV or TAV infected parents were divided into 3 variants each: i. Optimal variant (O): plants maintained under optimal temperature (27/21^{0}C day/night) and water regime; ii. Drought (D) - plants exposed to water deficit by restricting the water regime until leaf wilting, maintained at 27/21^{0}C (day/night) with application of stress in 3 rounds; iii. Heat stress (H) - plants exposed to a progressive temperature increase up to 42/25^0 C (day/night) for 7 days under optimal water regime, air humidity about 65-70% in the climate chamber. Biomorphological assessments were performed 14 days after stress initiation, 7 days of stress and 7 days of post-stress rehabilitation.

Pollen. Plants of different variants (Control, TMV, TAV) were grown under solar conditions during May-July and served as pollen donor. Heat stress for pollen was modelled *in vitro*, by applying temperature of 40^{0}C for 3 h, and drought stress was simulated by supplementing the culture medium with 35% sucrose (27^{0}C – as in control). The indices were evaluated based on microscopic studies.

RESULTS AND DISCUSSIONS

Sprouts. The research established the existence of morphological peculiarities in tomato sprouts when the seeds derived from plants infected with TMV or TAV compared to those from healthy plants. The results were confirmed in repeated experiences the particulars by seeds germination rate, the radicle or sprout length were recorded even four generations after the action of the viral factor.

The mean values of radicle length varied widely depending on genotype, phytosanitary status of the seed and growing conditions. Thus, heat stress decreased root growth intensity by 1.9-3.4 (except Mary Gratefully) compared to optimal conditions in control variants (from healthy plants), and for drought the decrease amounted to 1.1-3.3-fold (*figure 1 A*). The progeny of the first generation from TMV and TAV-infected plants also reacted differentially to heat stress, so that for Rufina genotype the decrease in root growth intensity compared to optimal conditions was 1.9 and 4.6-fold, respectively, for Mary Gratefully 3.4 and 1.4-fold and for Flacara 3.0 and 1.75-fold. Under optimal conditions in 4 out of 6 analysed genotypes, higher values for radicle growth intensity were established compared to the control in at least one of the TMV or TAV variants, the exceedance constituting 8-150% (8, 35, 150, 21), only for Rufina and *S. pimpinellifolium* control indicating higher values for this index. The response was also differentiated between control and TMV or TAV variants.

The ratios of intensity of root growth under abiotic stress and optimal conditions result in the index of resistance to the stress factor, which according to the data in the diagram denotes a broad picture of reactions depending on the sum of factors, stress-health status, expressed by specific genotypes with the highest indices of resistance under drought stress for the control variants (Mary Gratefully, Flacara, *S. pimpinellifolium*, Rufina, Venet and Tomis) vs. heat stress (Mary Gratefully, Flacara, Tomis, *S. pimpinellifolium*, Rufina, Venet) are very similar, except for the Tomis genotype (*figure 1 B*). At the same time we note that the reactions of the genotype depending on the applied stress. We note that in general the ranking of

TMV or TAV variants usually showed resistance index values different from the control, higher or lower values, for both types of stress, as well as in some cases differences between TMV or TAV variants (D - Tomis, SP; H - MG, Rufina, Flacara) were attested.

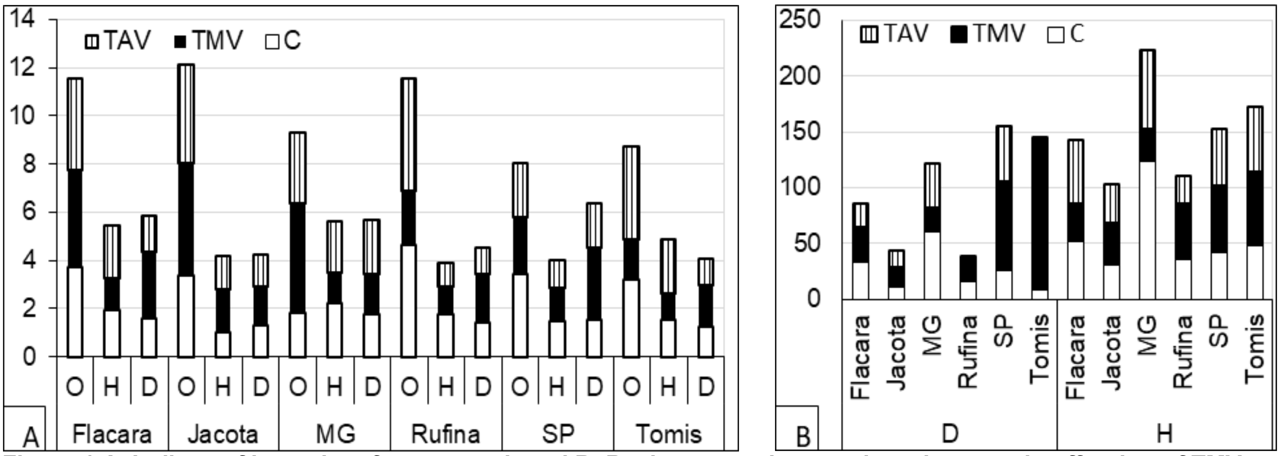

Figure 1 A Indices of intensity of root growth and B. Resistance **to heat or drought stressin offspring of TMV, TAV or control (C) variants, %. O - Optimal condition, H - heat stress, D - drought stress; MG - Mary Gratefully, SP - *S. pimpinellifolium*.**

In the same conditions, for the genotypes Mary Gratefully, *S. pimpinellifolium* and Tomis, great differences in drought stress resistance values were established between the variants derived from TAV or TMV infected plants and the control. However, the TMV variants of *S. pimpinellifolium* and Tomis genotypes significantly outperformed the resistance indices compared to the control, indicating a better adaptability of these variants to water deficit. These results correlate with higher radicle elongation for the given variants under stress conditions compared to the other analysed variants. At the same time the variants from healthy (control) plants of Mary Gratefully showed much higher heat resistance index values compared to the variants from infected plants. The analysis of the heat stress resistance indices revealed that TMV variants of Rufina, *S. pimpinellifolium* and Tomis genotypes showed slightly higher values compared to the control and TAV variants, but within the same resistance group.

Evaluation of architectural traits in offspring of infected plants under heat or water stress. The evaluation of plant response to stress can be performed by a variety of physiological, biochemical, morphological indices; however, the most eloquent index is productivity and biomorphological parameters of plants. Under optimal or heat stress conditions the values of the plant height were significantly higher in the control plants than in the TMV and/or TAV variants (derived from TMV or TAV infected plants), with the exception of the Rufina genotype, while under drought stress conditions the control variants, on the contrary, showed significantly lower values than at least one of the TMV or TAV variants. We also note that drought stress suppressed more strongly the indices of the plant height, leaves length and number than high temperature for a considerable part of the analysed variants. For example, plant height exhibited significantly lower values in the variants exposed to drought compared to heat stress for all control, TMV and TAV variants of genotypes Rufina and Mary Gratefully, and control and TMV for Jacota, as well as the control variant *S. pimpinellifolium.*

The analysis of variance allowed to establish that the highest contribution to the variability in the manifestation of the studied traits is due to *genotype* (14…24%), interaction of the factors *health status* and *stress* (7…19%), followed by the *stress* (3…18%) (*figure 2 A*). However, some specific features of the stress response can be deduced from the dispersion analysis for each genotype. According to the obtained results we can assume that the greatest contribution to the variability of the plant height trait is due to *stress* and the interaction of *stress x health status* (*figure 2 B*). Thus, the values of the contribution of *stress* in the variability of the plant height are 22…32%, followed by the interaction of *stress* x *health status* factors 13…28%. At the same time, we find, that the *health status* had the highest impact on plant height for *S. pimpinellifolium* - 15%, the lowest for genotype Jacota - 9% and insignificant for Mary Gratefully and Rufina. For *S. pimpinellifolium* the highest contribution indices for each factor analysed per experiment were attested.

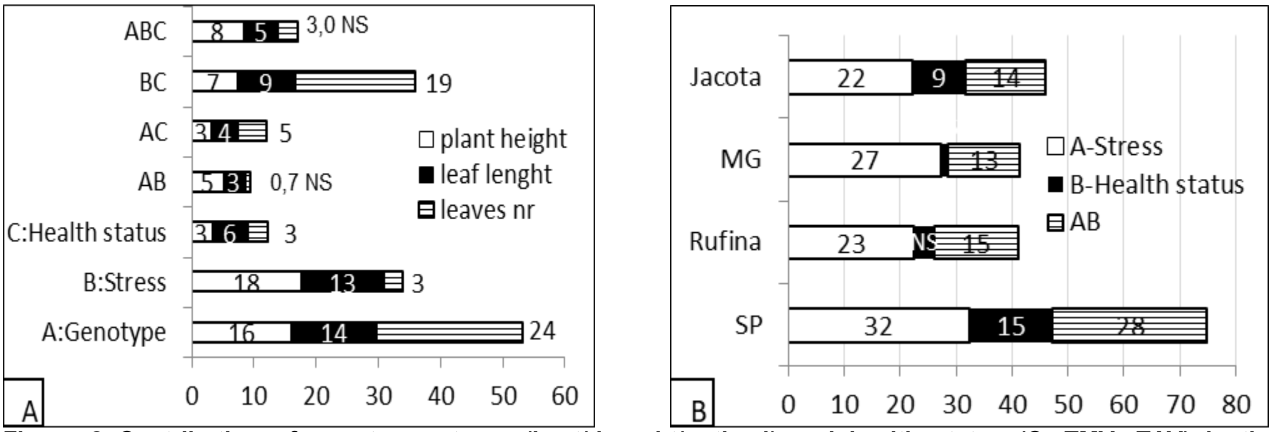

Figure 2 Contribution of genotype, stress (heat/drought/optimal) and health status (C, TMV, TAV) in the variability of traits %: A. Plant height, length and number of leaves; B. Plant height variance in dependence on genotype. All interpretation based on ANOVA analysis. NS - not significant, in rest of cases significant for P≤0.001. MG - Mary Gratefully, SP - *S. pimpinellifolium*

At the same time a differential response of genotypes of different health status depending on the applied stress was attested (*table 1*). Thus, the *genotype* has the greatest effect on the variability of the plant height under both heat and drought stress (17.5% and 22.2%), while the factors *health status* and *stress* have a diametrically opposite effect depending on the type of applied stress, so that *drought stress* has an effect of about 18.5% in the variability of the trait, and against the background of heat stress plant *health status* determined 11.6% of the variability. The interaction of the combinations of analysed factors conditioned a proximate variation similar to the type of stress applied (2.6-5.8%).

Heat stress or drought stress caused specific reactions within the genotype depending on the health status (Control, TMV, TAV), according to the differences between the mean values of the plant height and the dispersion analysis for each type of stress separately established that the interaction of the factors considered *genotype-health status-stress* had a significant impact on the variation of the trait, but not heat stress as a solitary factor (*table 1*).

Table 1

Analysis of variance of plant height according to the type of applied stress

Source of variance		Df	Heat stress			Drought stress		
			Sum of Squares	F-Ratio	Factor Contribution, %	Sum of Squares	F-Ratio	Factor Contribution, %
A: Genotype		3	1888.22	24.31***	17.5	2804.69	40.70***	22.2
B: Stress		1	35.12	1.36	0.3	2334.06	101.60***	18.5
C: Health status		2	1258.32	24.30***	11.6	354.16	7.71***	2.8
Interactions	AB	3	773.29	9.95***	7.2	323.09	4.69**	2.6
	AC	6	833.21	5.36***	7.7	699.19	5.07***	5.5
	BC	2	286.39	5.53**	2.6	620.22	13.50***	4.9
	ABC	6	765.01	4.92***	7.1	1069.07	7.76***	8.5
Residual		192	4971.48			4410.69		
Total		215	10811.0			12615.20		

*****, ** - significant for P≤0.001 and 0.01**

Pollen. The results of our research on male gametophyte variability revealed that temperature significantly impacted pollen viability, reducing it by 1.6 to 2.0 times in both healthy plants and progeny of TMV/TAV-infected plants, respectively. Additionally, TMV/TAV progeny exhibited a 28.4% to 32.9% reduction in pollen tube length compared to the control, potentially due to a slower growth rate of pollen tubes in these genotypes. Based on pollen grain distribution by pollen tube length under optimal conditions in Mary Gratefully TMV/TAV and *S. chilense* TAV progeny, it was established that the frequency of pollen grains, which formed pollen tubes of large size, increased, exceeding the control. Under water deficit background in most cases pollen grains formed short or medium sized pollen tubes, which confirms the negative action of drought stress on the germination and growth of pollen tubes. To identify the factors determining the variability of functional traits in pollen, the obtained results were processed using a three-factor analysis of variance (*table 2*).

Table 2

Analysis of variance for pollen traits in heat stress conditions *in vitro* in offspring of TMV and TAV plants

Source of variance		Polen viability		Pollen tube length	
	Df	Sum of Squares	Factor contribution, %	Sum of Squares	Factor contribution, %
A: Genotype	7	1226.9*	5.6	1323.2*	17.9
B: TMV	1	631.9*	2.9	473.9*	6.4
C: Stress	1	17748.0*	81.5	3056.7*	41.3
Interactions: ABC	22	2169.0*	9.92	2543.1*	34.3
Residual	64	2.92*	1.3	2.93	0.04
A: Genotype	7	1893.7*	7.9	1495.4*	19.7
B: TAV	1	945.6*	3.95	2799.4*	36.8
C: Stress	1	18931.0*	78.1	1646.7*	21.7
Interactions: ABC	22	2172.0*	9.1	1653.9*	21.8
Residual	64	2.96*	1.1	2.72	0.04

***- significant for P≤0.05**

The analysis confirmed the significant influence of *genotype* (5.6...19.7%), *heat* (21.7...81.5%), *drought* (55.4...82.1%, not presented here), *TMV/TAV* (2.9…36.8%) and their interactions on the variability of male gametophyte traits. Among these, the contribution of *heat* to the overall structure of pollen viability variability was decisive, accounting for 78.1 and 81.5%, while the effects of other factors, including *genotype*, were significantly weaker. The analysis of variability in pollen tube lengths of *TMV* progeny revealed that 41.3 and 34.3 % of the length variability was determined by temperature and factor interactions, respectively. In TAV progeny, the influence of *genotype*, heat *stress* and factor interactions on this trait was approximately equal, ranging from 19.7% to 21.8%, while the effect of the virus was stronger at 36.8% (*table 2*). Thus, heat stress is the main factor determining the variability of pollen viability in the TMV/TAV offspring, whereas the variability in pollen tube length, depending on the virus type, is largely determined by the influence of heat stress or TAV.

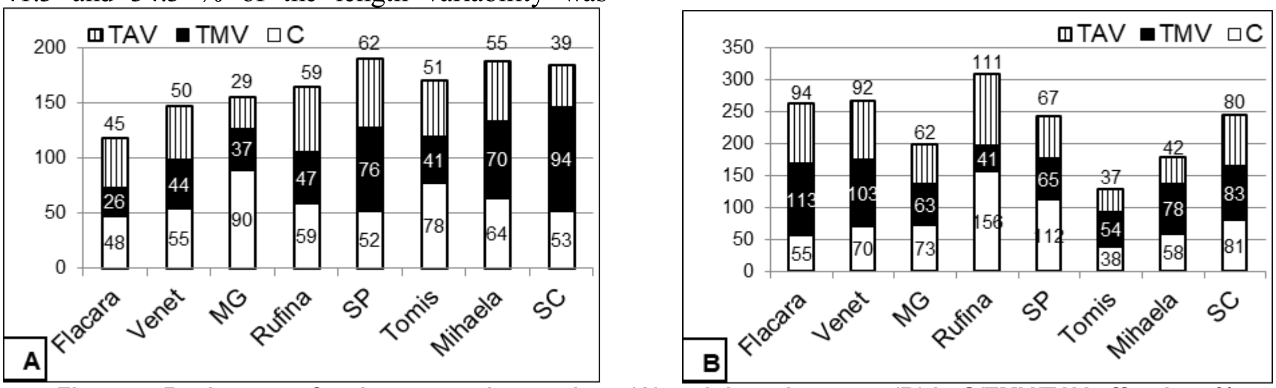

Figure 4. Resistance of male gametophyte to heat (A) and drought stress (B) in C/TMV/TAV offspring, %
MG - Mary Gratefully, SP - *S. pimpinellifolium*, SC - *S. chilense*.

The estimation of male gametophyte heat stress tolerance in control (offspring of healthy plants) and offspring of infected plants established that the plants in control variants showed the highest level of values of this trait - 62.5% (per experience), although in the TMV/TAV offspring, the level of thermoresistance was lower by 13.3 and 22.2%, respectively (*figure 4 A*). Based on the generalization of the obtained data, it was found that the male gametophyte in the control plants and progeny of the infected variants for Mihaela and Rufina, as well as the *S. pimpinellifolium* combine a high level of pollen heat resistance at the germination and pollen tube growth stage, which implies the possibility of their use in breeding research. Under conditions of drought (*figure 4 B*), three varieties: Venet, Flacara and Rufina, as well as the *S.pimpnellifolium* and *S.chilense*, were highlighted by their high pollen resistance.

CONCLUSIONS

Differential manifestation of some biomorphological traits at sporophyte (sprouts and plants) and gametophyte stage in progenies from TMV and TAV -infected vs. healthy plants under heat or drought stress conditions was confirmed, indicating bidirectional variations in mean values. For certain genotype-stress-health status combinations, better values were established under stress conditions compared to the control, indicating a better adaptability of these variants to stress factors. At the plant level variability of evaluated quantitative traits was conditioned by genotype (14…24%), stress (3…17%) and plant health status (3…6%).

The action of abiotic factors (heat/drought) on male gametophyte in offspring of virus infected plants causes differential changes in pollen functional characters, which are determined by genotype (5.6...19.7%), heat (36.8...81.5%), drought (55.4...82.1%) and health status (2.9…36.8%).

Based on the complex approach of gametic selection methods and genetic-statistical analysis, the structure of the variation spectra of male gametophyte indices in offspring of virus infected plants under heat or drought conditions was elucidated, which allows applying the results to predict the response of genotypes to the action of abiotic factors; genotypes with high degree of resistance of male gametophyte to the action of abiotic factors were highlighted.

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