

## STUDY OF TOLERANCE TO *TOMATO SPOTTED WILT VIRUS* IN TOMATO GENOTYPES WITH ANTHOCYANINS CONTENT

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### Abstract

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*Tomato spotted wilt virus* (TSWV) is one of the ten most widespread plant viruses in the world that causes diseases strongly affecting plant development and yield. The objective of the study was to determine the tolerance to TSWV of four tomato genotypes differing in anthocyanins content in their leaves and stems. The percentage of symptomless healthy plants among the four genotypes varied as follows: Keti (AA1) – control – 21% symptomless healthy plants; line St 993 x Keti (AA7) – 5% symptomless healthy plants, AA10 (VK1 x Keti) – 31% symptomless healthy plants and AA12 (VK1 x Keti) – 25% symptomless healthy plants. It is important to note that line AA10 was characterized by high productivity and tolerance to environmental conditions. No correlation was detected between the high content of anthocyanins in plant tissue and the high tolerance to TSWV.

*Key words:* *Tomato spotted wilt virus*; TSWV; tomato; tolerance; anthocyanins

### Introduction

*Tomato spotted wilt virus* (TSWV) is one of the most important agents of virus disease affecting tomatoes, in some cases leading to losses of up to 100%. TSWV is one of the most wide spread plant viral pathogens on vegetables tomato and pepper in particular, flowers and aromatic and medicinal plants in the world (Mavric and Ravnikar, 2001; Soler et al., 2003; Tomassoli and Barba, 2005; Dikova, 2011, 2014). Genetic resistance appears as the best solution to control this disease (Rosello et al., 1996). Some of the specimens of *Lycopersicon esculentum* contain Sw-5 gene for resistance against TSWV and other genes – Sw1, Swb1, Sw2, Sw3 and Sw4 (Czech et al., 2003; Ciuffo et al., 2005; Saidi and Warade, 2008). Stevens et al., 1991 established the inheritance of TSWV resistance due to a single

dominant gene. The SA, F1 and the backcrosses to the resistant parent were found to have eight out of 612 tomato plants infected four months after the inoculations, which indicates a 98.7% penetrance of the resistance gene (Stevens et al., 1991) Sometimes by reason of an appearance of a strong virulent strain of TSWV an interruption appears in the resistance of genotypes, consisting Sw 5 gene. (Aramburu and Marti, 2003). It is known that the anthocyanins have important protective role against abiotic stress conditions, pathogens and insects. According to Heim et al. (1983) prominent zones of intense anthocyanin accumulation often surround restricted lesions where often a plant disease pathogen has been successfully contained.

The objective of the study was to determinate the tolerance to TSWV of four tomato genotypes differing in anthocyanins content in their leaves and stems.

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## Materials and Methods

Four tomato genotypes with different content of anthocyanins were used: Line AA1 was characterized by highest anthocyanins content in plants leaves and stems, followed by AA7 and AA12, while line AA10 was characterized by lowest content of anthocyanins (Figure 1). The plants from each genotypes were cultivated in iron-sheet containers with size (length: width: height = 40/40/15 cm) in three repetitions in glasshouse conditions.

The indicator method by Noordam (1973) was used for obtaining and multiplying of the virus isolate, descending from pepper on the test tobacco plants from the species *Nicotiana tabacum* cv. Samsun NN as well as for inoculation of the tomato plants from the four genotypes. Tomato plants were mechanically inoculated in two – four leaves stage and after two weeks were observed for TSWV symptoms.

The pepper isolate was obtained after triple passages on the test plant *Chenopodium quinoa* from single chlorotic local lesion. We used pepper isolate of TSWV because in many cases this virus is in individual infection in pepper fruits and rarely in mixed infection with *Cucumber mosaic virus*

(CMV) or *Tomato mosaic virus* (ToMV) as in tomato fruits.

Each sample from an individual tomato plant was analyzed by us using ELISA method (DAS – ELISA); (Clark and Adams, 1977) with a kit for TSWV, purchased from the German company LOEWE, Biochemica. So from genotype AA1 were analyzed 19 plants, from genotype AA7 – 20 plants, from genotype AA10 – 26 plants and from genotype AA12 – 20 plants. We have determined the healthy plants, the latent virus carriers and the diseased plants from each genotype.

The extinction values were measured using a spectrophotometer Multimode Detector DTX 880. All samples showing values two and a half times higher than the negative controls were assumed as virus positive i.e. virus carriers. Negative controls were samples of symptomless healthy tomato plants and positive controls – TSWV infected indicator plants as well as the positive control from the kit.

The extinction values (the optical density) of the samples were processed by statistical analysis of Student's criterion, quoted by Lidanski (1988). Average extinction values of optical density were calculated as well as standart deviation. The confidence intervals are at a significance rate of  $p \leq 0.05$  at Student's criterion (Table 1).

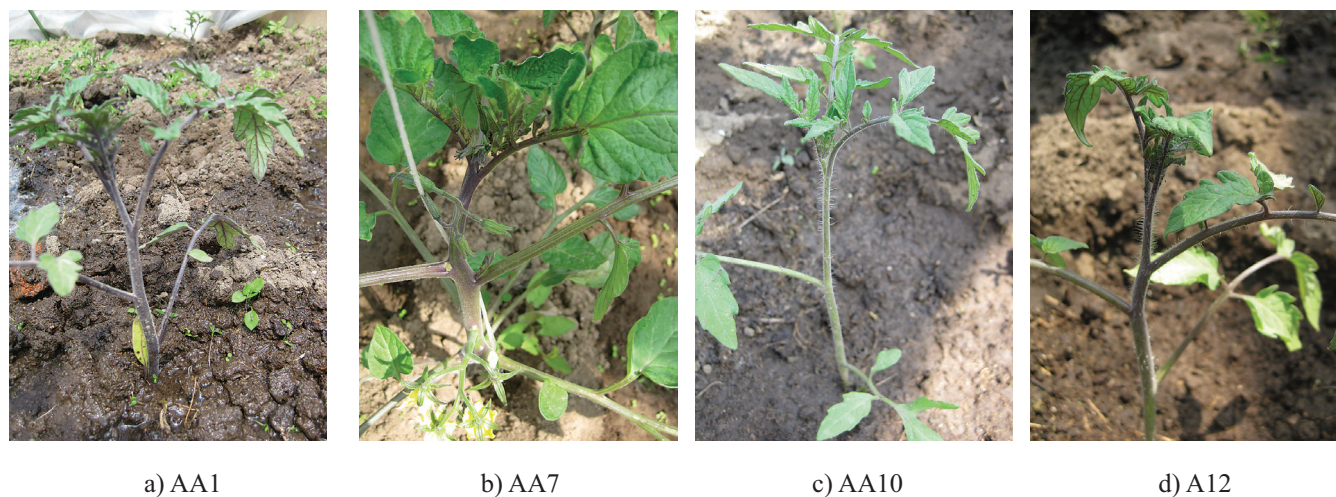


Fig. 1. The content of anthocyanins in tomato plants: a) AA1; b) AA7; c) AA10; d) AA12

Table 1

Results from tolerance to Tomato spotted wilt virus (TSWV) of tomato genotypes

Genotype	Tested plants (number)	Infected plants			Symptom less plants		
		Number	%	OD	Number	%	OD
AA1	19	15	79.0	0.628 ± 0.09	4	21.05	0.104 ± 0.02
AA7	20	19	95.0	1.019 ± 0.17	1	5.00	0.104 ±
AA10	26	18	69.2	0.539* ± 0.12**	8	30.8	0.094 ± 0.03
AA12	20	15	75.0	0.438 ± 0.07	5	25.0	0.119 ± 0.02

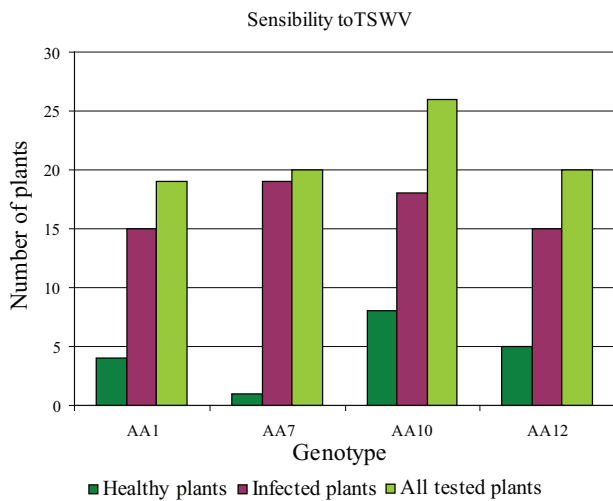
OD (Optical density)

Confidence intervals and \*\* – standard deviations at  $P \leq 0.05$  by Student's criterion

## Results and Discussion

*Tomato spotted wilt virus* (TSWV) causes symptoms of chlorotic turning in necrotic spotting on the leaves of the tomato plants from the fourth genotypes. Symptoms of mosaic were observed on the top leaves of strongly susceptible plants, belonging to the fourth genotypes, but the tolerant and resistance plants from each genotypes were without mosaic symptoms. Dwarfing by reason of shortening of internodes of the infected plants was observed in some cases.

Genotypes AA10 and AA12, both VK1 x Keti had the highest percentage – the first 30.8% healthy plants (Table 1, Fig-



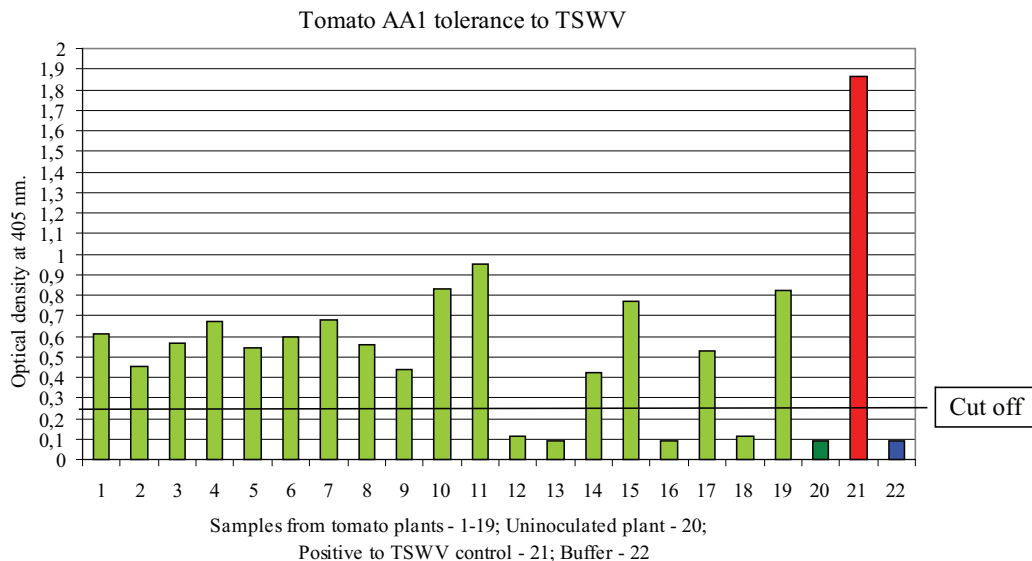
**Fig. 2. Tolerance to *Tomato spotted wilt virus* (TSWV) of tomato genotypes**

ure 2) and the second 25.0% healthy plants (Table 1, Figure 2) in comparison with the other genotypes. The TSWV tolerant plants from each genotype were with symptomless leaves in comparison with susceptible plants with symptoms of mosaic.

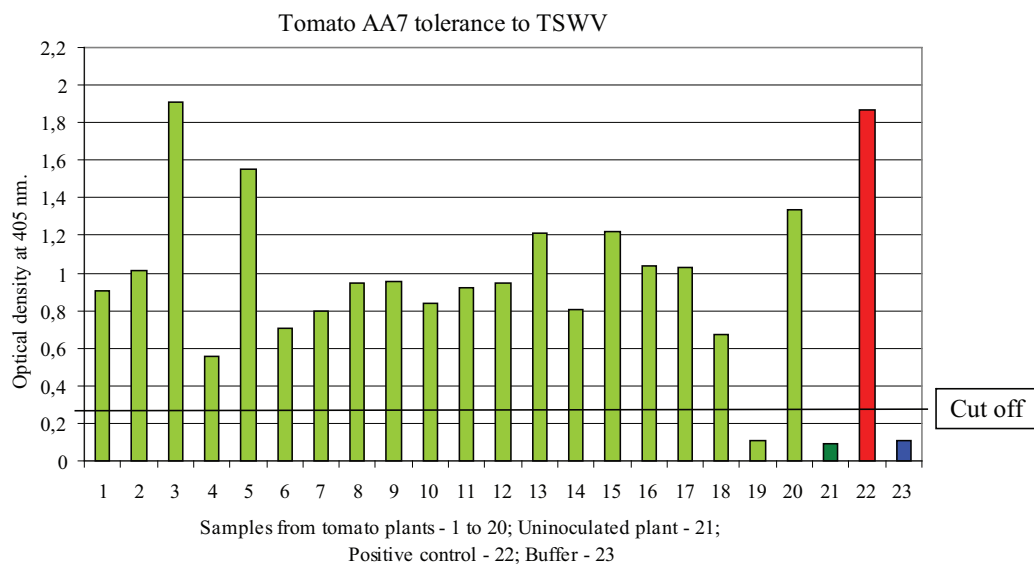
Four healthy plants with a confidence interval (an average extinction and standard deviation) –  $0.104 \pm 0.018$  were established in genotype AA1 (Keti), representing 21.05% (Table 1, Figure 3). Only one healthy plant from the genotype AA7 (St 993 x Keti), representing 5.0% was symptomless with an extinction value – 0.104 OD and the highest average extinction value in the confidence interval from the infected plants –  $1.019 \pm 0.174$  (Table 1, Figure 4). Eight healthy plants from the genotype AA10 (VK1 x Keti), representing 30.8% were symptomless and with negative extinction values, expressed by the confidence interval (an average extinction value and standard deviation) –  $0.094 \pm 0.033$  (Table 1, Figure 5).

A moderate average extinction value in the confidential interval –  $0.397 \pm 0.060$  OD was established for twelve tomato plants from genotype AA10. Many of these twelve plants were symptomless, but their tolerance was not reliable, because they were latent carriers of TSWV i.e. they were not immune. Six plants of genotype AA10 had a strong average extinction value in the confidential interval  $-0.823 \pm 0.197$  OD and they had severe symptoms of spotting and mosaic on the top leaves (Figure 5).

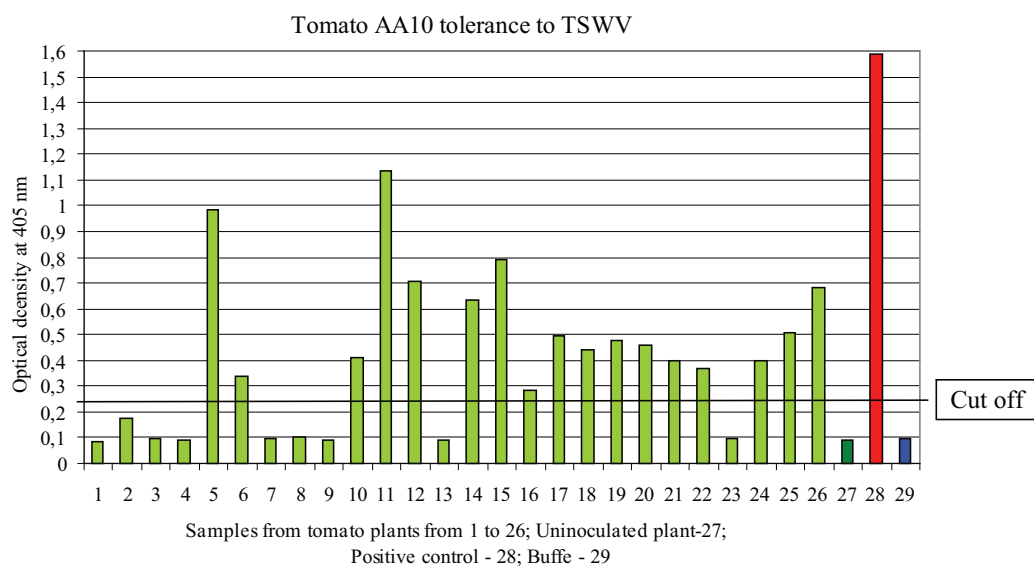
Genotype AA12 (VK1 x Keti) had five healthy plants with a confidence interval (an average extinction and standard deviation) –  $0.119 \pm 0.023$ , representing 25.00% and the lowest average extinction in the confidence interval of the TSWV infected plants in comparison with the other genotypes –  $0.438 \pm 0.072$



**Fig. 3. Data from DAS-ELISA for tolerance to TSWV of line AA1**



**Fig. 4. Data from DAS-ELISA for tolerance to TSWV of line AA7**



**Fig. 5. Data from DAS-ELISA for tolerance to TSWV of line AA10**

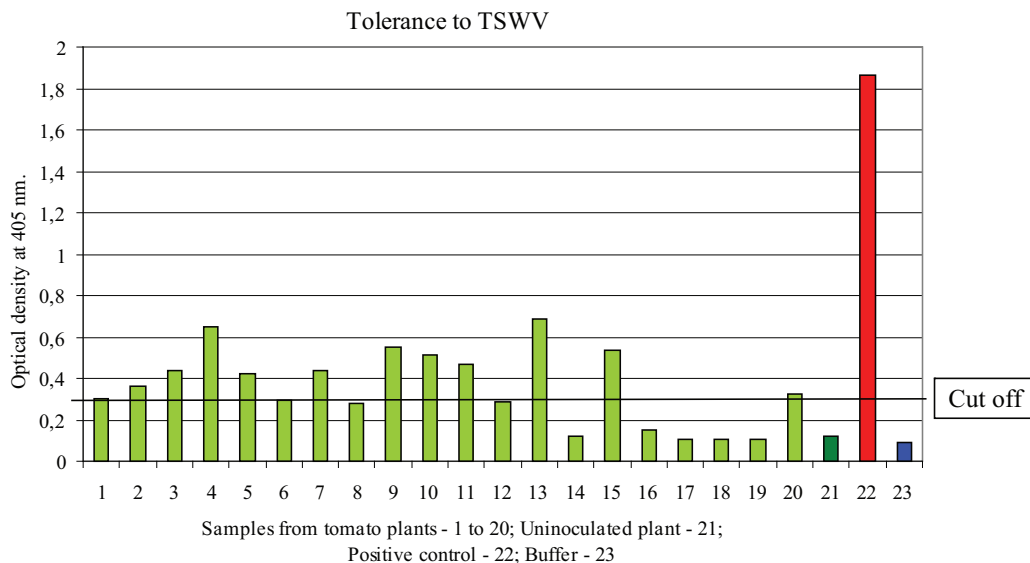
(Table 1, Figure 6). The presence of symptomless healthy plants might be due to the presence of gene/s, controlling the tolerance to TSWV.

It is known that the anthocyanins have important protective role against abiotic stress conditions, pathogens and insects. Line AA1 (Figure 1a) was characterized by highest anthocyanins content in plants leaves and stems, followed by AA7 (Figure 1b) and AA12 (Figure 1d), while line AA10 (Figure 1c) was characterized by the lowest content of anthocyanins.

It is important to note that line AA10 was characterized by

high productivity and tolerance to environmental conditions. The results obtained showed that no correlation was detected between the high content of anthocyanins in plant tissue and the high tolerance to TSWV. According to Hipskind et al., 1996 anthocyanin accumulation occurred considerably after the growth of the pathogen and had stopped in cells that were affected, but not infected by the pathogen.

But these data probably refer to bacteria and fungi, because for the plant viruses it is very important to search for the resistance genes.



**Fig. 6. Data from DAS-ELISA for tolerance to TSWV of line AA12**

## Conclusions

Three of four genotypes: AA10 (VK1 x Keti) with nearly 31% healthy plants; AA12 (VK1 x Keti) with 25% healthy plants and AA1 (Keti) with 21% healthy plants give reasons to continue the study on these genotypes. We expect AA1 (Keti) and AA12 (VK1 x Keti) that contain anthocyanins to be responsible for the creation of complex resistance to plant viruses, bacteria and fungi.

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