The Effects of *Tobacco mosaic virus* Infection on Growth and Physiological Parameters in Some Pepper Varieties (*Capsicum annuum* L.)

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Abstract

Changes in some growth (plant leaf number and area, plant biomass, plant height, root length, and plant stem diameter) and physiological (photosynthetic pigments, relative water content (RWC) and proline content) parameters of pepper (*Capsicum annuum* L.) varieties were studied as they were affected by *Tobacco mosaic virus* (TMV) infection. The greenhouse pepper cvs ‘Ergenekon F₁’, ‘Kumsal F₁’, and one candidate variety (‘497 F₁’) were used as material to compare infected versus non-infected control plants. Infected plants showed various degrees of stunting; necrosis on stems, leaves and fruits; mosaic symptoms on leaves; deformations, defoliation of leaves, and reduction in fruit size. Besides these, TMV infection resulted in reduction of vegetative growth parameters, RWC, chlorophyll a and b, plant fresh and dry production in different parts of the plants, but increase of proline content in leaves. The level of response differed depending on varieties.

Keywords: growth, pepper genotypes, physiology, TMV (*Tobacco mosaic virus*), virus

Introduction

*Tobacco mosaic disease* is one of the major limitations in production of pepper (*C. annuum*), an important vegetable crop in Turkey and all over the world, in both greenhouse and open field. This disease, caused by *Tobacco mosaic virus* (TMV, genus: Tobamovirus), a positive-sense single stranded RNA virus, transmits easily through seed, soil and cultural processes, and can cause important losses of yield and quality properties (Scholthof *et al.*, 1999). Virus-infected pepper plants typically develop symptoms including various degrees of mosaic symptoms, chlorosis on leaves, stems, and fruits, necrosis on several plant parts, and stunting (Baker and Adkins, 2000). All the various macroscopic and microscopic symptoms of diseases must originate in biochemical aberrations induced directly or indirectly by the virus (Hull, 2009).

To date, several studies have focused on interactions between different plants and virus combinations. The level of total chlorophyll (Chl) and carotenoids (Car) in control and *Yellow vein mosaic virus* (YVMV) infected leaves was studied by Palanisamy *et al.* (2009), who reported that total Chl and Car concentrations were significantly reduced in infected leaves by 64% and 62%, respectively. It was recorded that *Zucchini yellow mosaic virus* (ZYMV) infected leaves of pumpkin showed severe symptoms as mosaic, green blisters, size reduction and deformation and the virus infection diminished the Chl a (48%), Chl b (53%) and carotenoid contents (52%). Nevertheless proline contents of ZYMV infected plants were found higher than controls (Radwan *et al.*, 2007). *Tobacco ringspot virus* (TRSV) infection did not significantly affect the number of *Lotus corniculatus* roots produced on the stem cuttings in 14 days; however virus infection significantly reduced the length of the longest root. Also significant yield reduction was observed (Ostazeski, 1970).

As mentioned above there are number of papers that studied the results of different virus-plant combinations, however there is little known about TMV effects on growth and physiological parameters of pepper. The objectives of this study were to clarify the effects of virus infection on host growth and physiological parameters, and to observe the reactions of pepper varieties under TMV disease conditions.

Material and methods

**Growing conditions and plant materials**

This experiment was carried out in greenhouse conditions at Faculty of Agriculture Ege University (27,09° E, 38,25° N), Turkey in the autumn season of 2011. The greenhouse pepper cvs ‘Ergenekon F₁’ (bell pepper type, Bircan Tarım, Antalya-Turkey), ‘Kumsal F₁’ (Banana type, Bircan Tarım, Antalya-Turkey), and one candidate variety (‘497 F₁’) (Banana type, Bircan Tarım, Antalya-Turkey) were used as plant material. Seedlings of peppers (*Capsicum annuum* L.) were transferred in a mixture of soil, farm manure, silt and peat (1:1:1:1) in sterilized plastic
pots (200 × 180 millimeter) on October 17, 2011. All cultivars and candidate variety were grown in greenhouse until February 26, 2012 under maintained 20±5 °C, 60–80% relative humidity and standard day light conditions. All pots were irrigated with tap water every three days to maintain soil at the field capacity and all other cultivations were discharged uniformly throughout the whole experimental period.

**Pathogen inoculation**

The TMV used in this study is a common strain which induces mosaic containing light and dark-green areas in tobacco. The pepper leaves infected by TMV (provided by Plant Protection Department, Ege University, Turkey) were homogenized in 0.05 M phosphate buffer (pH 7.0) and used as inoculum. Plants at 4-6 fully expanded leaf stage were mechanically inoculated on the adaxial surface by using celite powder. The control group was also dusted with celite powder and washed with free inoculum water in order to provide the independence of error.

**Measured parameters**

**TMV damage scale class** - Plants were scored on the 35th and 70th days after inoculation (November 30, 2011 and January 03, 2012, respectively) for severity of TMV susceptibility by 1 to 5 scale modified by Leelerg (1967). Accordingly, (1) is scored as undamaged, healthy leaves or slightly involuted normal green leaves, (2) completely involuted green leaves, (3) moderately-excessively damaged dry leaves in addition to involuted green leaves, (4) (50 to 80%) drying damage on most leaves (50 to 80%), (5) drying damages on all leaves.

**Growth** - Four plants from each replicate were removed 133 days after transplanting in order to determine plant growth parameters. All leaves on the plants were counted and the mean values were given as pieces per plant ᵇ. Plant height was determined by measuring stem length from soil surface to the tip of the stem for all the plants and results were given cm⁻¹ plant⁻¹. Stem diameter (mm plant⁻¹) was measured on three parts of stem by digital caliper (Canon, 428830, China) and calculated with arithmetic mean. Following these measurements, harvested plants were separated into plant parts (root, leaves, stems and reproductive parts). The root systems were carefully washed with tap water to separate substrates. The longest root length (cm plant⁻¹) was measured as the distance from soil surface to the end of the longest root. All plant parts were weighted for fresh weight. Then, samples were dried at 65 °C in thermo ventilated oven for 72 hours and their dry weights were recorded. Results were presented as the dry and fresh weight (g plant⁻¹) of root and shoot. Tree plants of each treatment were used for total leaf area according to Öztekin and Tuzel (2011). The area of the mentioned leaves were measured by Sigma Scan Pro 5.0 (Automated Image Analysis, Systat Software Inc., USA) software and detected as pix² per plant. These data were converted into cm² per plant.

**Physiological parameters** - The relative water contents (RWC) of leaves were measured according to Smart and Bingham (1974). After sampling, leaf discs were taken and their fresh weight were immediately weighed and then immersed in distilled water for 7 h at room temperature. The leaves were then blotted dry and weighed prior to oven drying at 70°C for 48 h. The leaf relative content was calculated using the following formula:

\[ RWC(\%) = \frac{(FW-DW)}{(TW-DW)} \times 100 \]

FW, DW and TW are the fresh, dry and turgid weights of the tissue, respectively.

The procedure for chlorophyll determination was based on the work of Arnon (1949) on the absorption of light by aqueous acetone (80%) extracts of chlorophyll. The determination of chlorophyll a and b were made by measuring a 25 mg leaf, in density of 80% acetone (v/v) chlorophyll extracts with a Varian Carry 100UV-Visible spectrophotometer (Varian Inc, Palo Alto, CA, USA) at 450, 465 and 663 nm and setting up simultaneous equations using the specific absorption coefficients for chlorophyll a and b and carotenoid which were calculated with the following formula and were expressed in terms of mg per L fresh mass:

- Chlorophyll a = \((0.0127 \times 663nm) - (0.00269 \times 645nm)) \times 1000
- Chlorophyll b = \((0.0229 \times 645nm) - (0.00468 \times 663nm)) \times 1000
- Carotenoid = \((4.07 \times 450nm) \times ((0.0435 \times Cl a) + (0.367 \times Cl b))

Free proline content was determined according to Bates, et al. (1973). 0.5 g of leaf samples from each group were homogenized in 3% (w/v) sulphosalycylic acid and the homogenate was filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, resulting mixture was heated at 100 °C for 1 h in water bath. Reaction was then stopped by using an ice bath. The mixture was extracted with toluene and the absorbance of fraction with toluene aspirated from liquid phase was read at 520 nm. Proline concentration was determined using calibration curve and expressed as micromole proline per gram FW.

**Experimental design and statistics**

Analysis of variance (ANOVA) was carried out to determine statistically significant differences caused by infections in studied parameters. The experimental design was a completely randomized plots design with 4 replicates. Duncan’s Multiple Range Test was used to determine significant differences of means.

Results and discussion

While pathogens infect plant’s mineral uptake, depending on pathogen and on infected plant tissue, patho-
gens interfere with different physiological function(s) of the plant which leads to the development of different symptoms (Agrios, 2005). These alterations cause reduction in yield and quality of products. In this study, number of changes was observed in the physiology and morphology of the pepper plants associated with TMV infection. Our results have shown that the growth parameters such as plant height, root length, leaf area, biomass and physiological parameters such as total photosynthetic pigments, relative water content, were significantly decreased in virus-affected plants. These results were supported by other authors (Ostazeski et al., 1970; Kuhn and Dawson, 1973; Johnson and Main, 1983; Agrios et al., 1985; Funayama et al., 1997; Wintermantel, 2005; Taiwo and Akinjogunla, 2006). In addition, there were differences in the progress of TMV through different pepper varieties.

**TMV damages scoring**

The first symptoms of infection appeared on the uppermost younger leafs as necrosis and chlorosis along the main veins accompanied by wilting and leaf spots. Generally, infected pepper varieties were stunted, deformed, and they showed chlorotic areas among the leaves. These symptoms are in conformity with the symptoms observed by Rodriguez-Alvarado et al. (1994). The varieties responded differently to TMV as judged from their visual appearance. However, they showed similarities in the scale classes 0.2 to 4.0 on 35 days after inoculation and 0.4 to 4.8 on 70 days after inoculation. On November 30, 2011 the highest damage was observed with ‘497 F’ plants. A similar situation was also observed on January 03, 2012. Under short and long time stress conditions; ‘Kumsal F’, in scale class-1 were least affected by TMV treatment. Cv. ‘Ergenekon’ showed moderate tolerance to TMV according to their leaf damages (Tab. 1).

**Plant growth**

TMV infection decreased leaf number in all varieties (Tab. 2). While the leaf numbers of all varieties were nearly equal before inoculation, leaf number per plant was reduced significantly (p<0.01) by 42.05% for ‘Ergenekon’, 5.85% for ‘Kumsal’ and 59.87% for ‘497’ compared to the control. In virus-stressed plants, there were 57.51%, 3.88%, 83.56% decreases (p≤0.01) in leaf area in ‘Ergenekon’, ‘Kumsal’ and ‘497’, respectively, compared to the controls. Under our experimental conditions ‘Ergenekon’ and ‘497’ varieties were found to be affected more adversely than ‘Kumsal’ with regards to leaf number and leaf area.

TMV induced reduction in amount of leaf number and leaf area in all tested genotypes contributes to the diminished photosynthesis activity that is closely related with yield and quality of products. Similar results were obtained by Ehinmore et al. (2010) and Lehrer et al. (2007).

The virus infection caused a decrease (p<0.01) in plant height of ‘Ergenekon’, ‘Kumsal’, ‘497’, respectively (Tab. 3). ‘Kumsal’ gave the highest plant height compared the other varieties. The longest root length was decreased (p<0.01) by TMV infection (Tab. 3). Average decreases in root length of ‘Ergenekon’ (26.14%) caused by virus were less than that of ‘497’ (35.66%). The average longest root length for KUMSAL had an unexpected increase (reduced 5.94%) in virus infection (p<0.05). Contrary to

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**Tab. 1. Leaf damages according to TMV scale**

<table>
<thead>
<tr>
<th>Varieties</th>
<th>30.11.2011 **</th>
<th>03.01.2012 **</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Kumsal’</td>
<td>0.2 b</td>
<td>0.4 a</td>
</tr>
<tr>
<td>‘Ergenekon’</td>
<td>2.6 b</td>
<td>3.0 b</td>
</tr>
<tr>
<td>‘497’</td>
<td>4.0 b</td>
<td>4.8 a</td>
</tr>
</tbody>
</table>

**p<0.01**

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**Tab. 2. Effect of TMV infection on leaf number and leaf area of peppers**

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Leaf number (pieces plant-1)**</th>
<th>Leaf area (cm² plant-1)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Infected</td>
</tr>
<tr>
<td>‘Ergenekon’</td>
<td>25.17</td>
<td>14.58</td>
</tr>
<tr>
<td>‘Kumsal’</td>
<td>28.50</td>
<td>26.83</td>
</tr>
<tr>
<td>‘497’</td>
<td>25.33</td>
<td>10.17</td>
</tr>
</tbody>
</table>

**p<0.01, [-] shows decrease**

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**Tab. 3. Effect of TMV infection on plant height, the longest root length and stem diameter of peppers**

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Plant height (cm plant-1)**</th>
<th>The longest root length (cm plant-1)**</th>
<th>Stem diameter (mm plant-1)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Infected</td>
<td>Change (%)</td>
</tr>
<tr>
<td>‘Kumsal’</td>
<td>24.88</td>
<td>22.00</td>
<td>-11.55 a</td>
</tr>
<tr>
<td>‘497’</td>
<td>19.83</td>
<td>15.30</td>
<td>-23.10 b</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, [-] shows decrease, (+) shows increase**
Tab. 4. Effect of TMV infection on root and shoot dry and fresh weight of peppers

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Root</th>
<th>Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry weight (g plant(^{-1}))*</td>
<td>Fresh weight (g plant(^{-1}))**</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>TMV</td>
</tr>
<tr>
<td>Ergenekon</td>
<td>0.87</td>
<td>0.47</td>
</tr>
<tr>
<td>'Kumsal'</td>
<td>0.71</td>
<td>0.68</td>
</tr>
<tr>
<td>'497'</td>
<td>0.65</td>
<td>0.35</td>
</tr>
</tbody>
</table>

*Results show statistically significant differences (p<0.01) compared to control plants in 'Kumsal.'

In virus disease conditions reduction of shoot and root dry weight were statistically significant (p<0.01) (Tab. 4). However, the highest reduction in root dry weight (49.50%) was observed in '497' followed by 'Ergenekon' (45.37%). Root dry weight of infected 'Kumsal' plants was reduced only by 4.49%, which was significantly lower than those of other varieties. There were 43.40%, 52.13%, 10.69 decreases (p<0.01) in root fresh weight in 'Ergenekon', '497', 'Kumsal' respectively in virus-stressed plants as compared to the controls. The results show that TMV caused reduction in shoot fresh and dry weight (p<0.05). Both parameters were affected similarly and '497' showed the most, whereas 'Kumsal' showed the least reduction in shoot fresh and dry weight. The findings of Wani et al. (1991), Friess and Maillet (1997), Funayama et al. (1997), Pacheco et al. (2003), Wintermantel (2005), Kazinczi et al. (2006), Kollmann et al. (2007) on Ispoustrate these results.

Physiological Parameters

Relative water content in the leaves of plants grown under TMV infection stress decreased significantly in all cultivars and candidate varieties compared to uninfected plants (p<0.05) (Tab. 5). '497' showed greater reduction in the RWC than 'Ergenekon' (3.09%) and 'Kumsal' (2.12%). Similar results found by other authors (Synková et al., 2006; Al-Saleh et al., 2007).

Total proline content results are given in Tab. 6. These results show that leaves from all varieties subjected to TMV infection had increased proline content relative to leaves from control plants. Leaves of 'Kumsal' plants showed proline concentration increases of 0.88% compared to controls, whereas 2.97% and 4.89% increases in proline content were observed in leaves from 'Ergenekon' and '497' respectively.

Tab. 5. Effect of TMV infection on relative water content of peppers

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Relative Water Content (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Ergenekon</td>
<td>90.25</td>
</tr>
<tr>
<td>'Kumsal'</td>
<td>89.53</td>
</tr>
<tr>
<td>'497'</td>
<td>89.20</td>
</tr>
</tbody>
</table>

*Results show statistically significant differences (p<0.01) compared to control plants in 'Kumsal.'

Proline accumulation is a common metabolic response to both abiotic and biotic stress and when higher plants are exposed to stress; many plants accumulate high amounts of proline in tissues (Mansour, 2000; Mazid et al., 2011). In addition some other amino acids can also be found at higher concentrations (Drossopoulos et al., 1985). For instance, water deprivation (Ain-Lhout et al., 2001), salinity (Jouve et al., 2004), high and low temperatures (Lv et al., 2011), pathogen infection (Fabro et al., 2004) may cause activation in numerous compounds in the cell or the proline production (Verbruggen et al., 1993; Yoshida et al., 1995). In our experiment, a higher amount of proline in diseased material was observed compared to the respective controls in all genotypes (Tab. 6). The results are supported by Kakani et al. (2001), Liu et al. (2001), Radvan et al. (2007), Chatterjee and Ghosh (2008), Mohamed (2011). When plants are exposed to microbial pathogens, they produce reactive oxygen species (ROS) that induce programmed cell death in the plant cells surrounding the infection site to effectively wall off the pathogen and terminate the disease process (Apel and Hirt, 2004; Adi et al., 2012). The amino acid proline may act as a potent scavenger of ROS and this property of proline might prevent the induction of programmed cell death by ROS (Chen and Dickman, 2005).

The changes in the level of total chlorophyll (Chl) and carotenoids (Car) in control and TMV infected leaves are shown in Tab. 7. When determined on the basis of unit fresh weight, the Chl a and Chl b concentrations in infected leaves were significantly reduced in all genotypes (p<0.01).

Chlorophyll a and b contents of infected leaves were more than half of those of uninfected leaves in 'Ergenekon' (54.13%) and '497' (60.08%) genotypes. Kumsal showed less reduction of Chl (9.82%) among the other genotypes. From the data taken, and through analysis, we calculated that TMV infection reduced chlorophyll contents by
### Tab. 7. Effect of TMV infection on chlorophyll a, chlorophyll b and carotenoid of peppers

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Chlorophyll a (mg/L)**</th>
<th>Chlorophyll b (mg/L)*</th>
<th>Carotenoid (mg/L) NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Infected</td>
<td>Change (%)</td>
<td>Control</td>
</tr>
<tr>
<td>'Ergenekon'</td>
<td>3.85</td>
<td>1.77</td>
<td>0.72</td>
</tr>
<tr>
<td>'Kumsal'</td>
<td>3.13</td>
<td>2.82</td>
<td>0.57</td>
</tr>
<tr>
<td>'497'</td>
<td>3.73</td>
<td>1.49</td>
<td>0.77</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, NS: Not significant, (-) shows decrease

about 18.02%, 40.86%, 84.26% in 'Ergenekon,' 'Kumsal' and '497' compared to those of control group. The variance analysis of the carotenoid content showed that this particular TMV infection did not affect the carotenoid content. There are various theories about how Chl a and Chl b contents in infected leaves were decreased. According to Mandahar and Garg (1972) and Roca and Minguez-Mosquera (2003) decreases of chlorophyll was the result of increased chlorophyllase activity. On the other hand Lucas (1998) thought that it could be due to the blocking Chl synthesis. Similar results for numerous host plants infected with different viruses were published by Christov et al. (2005), Radwan et al. (2007), Palanisamy et al. (2009) and Kyselakova et al. (2011). Carotenoid content was not affected by TMV and that shows differences with the results of carotenoid data published by Palanisamy et al. (2009). In addition a remarkable finding of this study is that TMV reduced leaf number and leaf area in all tested genotypes, contributing to the diminished photosynthesis activity (Tab. 2). Similar results obtained by Lehrer et al. (2007) and Ehinmore et al. (2010).

### Conclusions

Based on the results of the present study, leaf number and area, plant height, the longest root length, stem diameter, dry and fresh weights of root and shoot were reduced significantly when peppers were exposed the TMV infection. Because of the fact that plant leaf number and total leaf area are directly related with photosynthetic activities which provide energy for growth and defense against pests and diseases, reduction of these parameters caused decline in overall growth resulting in shorter plants, slender stems, and reduced biomass compared controls. TMV infection also reduced relative water content and photosynthetic pigments except carotenoids. Reduction in relative water content and root length indicates that water transport in the soil-plant-atmosphere and respiratory system of the plants were adversely affected. Results also show that leaves subjected to TMV infection had increased proline content as compared to the leaves of control plants. The variation in the degree of change of this parameter suggests that the extent of damage is not the same in all tested groups. In general, 'Kumsal' showed more vigor and 'Ergenekon' showed moderate tolerance depending on its own characteristics when TMV was inoculated. On the other hand the highest damage was observed with candidate variety '497 F1. It is possible to say that, Kumsal is the most tolerant genotype among the other tested groups.

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