

Combined effect of TYLCV infection and drought mitigates stress in tomato (*Solanum lycopersicum* L.) through the modulation of antioxidant enzymes

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Received: September 25, 2024; Received in revised form: November 12, 2024; Accepted: December 19, 2024

Plant viruses cause considerable losses in agricultural crops globally, reducing the yield and quality of agricultural goods. We conducted the study of antioxidant enzymes (APO, BPO) in a local Azerbaijani genotype (Shalala) subjected to combined effects of drought and TYLCV stresses. Virus inoculation provided by *Bemisia tabaci* – whitefly insects that feed on them, in a controlled environment. According to our results, high enhancements in APO and BPO activity occurred only under drought and only in TYLCV-infected tomato samples. As a result, we found that the Shalala genotype had higher levels of APO and BPO, although these levels were very low and barely different from the control ones.

Keywords: *Solanum lycopersicum* L., drought, tomato yellow leaf curl virus, *Bemisia tabaci*, combined stress, ascorbate-peroxidase, benzidine peroxidase

INTRODUCTION

Environmental stress can cause plants to change the way they use their resources like physical and chemical qualities (Agrell et al., 2005; Cui et al., 2012). Priming defense, being a physiological process, makes plants get ready to respond faster or more strongly to climate change and biotic stress (Frost et al., 2008). This process greatly changes how insects and plants interact when they are dealing with environmental stress, disease, and insect infestations (Sun et al. 2013, 2017). Viral infection, alongside abiotic stress, can also affect the way whiteflies, viruses and plants interact. Begomoviruses are the most detrimental group of plant viruses in warm places, are often accompanied by outbreaks of whiteflies, particularly tomato yellow leaf curl virus, which is one of the most destructive begomoviruses and has global distribution (Bilgin et al., 2008; Guo et al., 2017). Sun et al. (2017) found that TYLCV makes the Mediterranean (MED) whitefly stronger by hindering its JA defense pathway. The whitefly,

Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae), is a type of bug that can cause a lot of damage to plants. It sucks the sap from the plants and can also spread viruses to them. It can be found worldwide and is a massive problem for farmers (Stansley and Naranjo 2010). *Bemisia tabaci* MED has recently spread in China causing huge damage to crops by feeding on them and spreading TYLCV (Chu et al., 2010; Rao et al., 2011; Cui et al 2018; 2019).

Diseases caused by viruses harm tomatoes and cause big losses in production around the world just like various biotic stress. Whiteflies spread a kind of virus called geminiviruses (genus: *Begomovirus*) that affects tomato plants. This virus is a big problem for growing tomatoes in hot and humid areas. These viruses make plants sick in different ways. One disease they cause is called tomato yellow leaf curl disease (TYLCD), which damages tomato plants (Prasanna et al., 2015). Tomato yellow leaf curl is a harmful virus that affects tomatoes. In countries with tropical and subtropical climates, tomato crops have been

damaged a lot. TYLCV is common and can be found in many places where tomatoes are cultivated. (Michael et al., 2009; Navas-Castillo et al. 2011; Chen et al., 2016). When plants get a virus, their chemical composition changes to a great extent causing a decrease in quality and quantity of the crops. Different reports say that when a virus multiplies in a plant cell, it changes the plant's chemical composition and disrupts the physiological processes like photosynthesis, transpiration and respiration of the infected plants which affect the growth and yield (Tajul et al., 2011; El-DougDoug et al., 2014b). This can cause the infected plants not to grow well and not produce as much. Furthermore, it has been reported that figuring out what makes up cells in virus-infected plants is really important for understanding what the host cells are doing and how much damage the virus has caused. A viral infection makes cells leakier, causing them to lose water. This also tells us why the infected leaves have a cup shape, especially when the symptoms are very bad (Oleinikova et al., 1969).

According to Moshe et al., (2012), responsive oxygen species (ROS) rummaging components in plants include proteins such as superoxide dismutase (Grass), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and catalase (CAT). SODs act as the primary line of defense against ROS, dismutating superoxide to H₂O₂. APX, GPX, and CAT along these lines detoxify H₂O₂. Most anti-oxidative proteins were recognized in TYLCV-infected tomatoes. SODS, APX, thioredoxin peroxidase, ferredoxin-nitrite reductase were more abundant in susceptible than in resistant plants. Plant thioredoxins are the key factors in oxidative stress response. TYLCV immunization enhances the defense chemicals of tomato such as peroxidase, polyphenol oxidase (PPO) and phenylalanine ammonia-lyase. TYLCV increases the exercises of defense proteins and diminishes disease index considerably so as to extend tomato resistance to TYLCV (Li et al., 2012; Sofy et al., 2014, 2017).

MATERIALS AND METHODS

Plant material and stress treatment: We used the Shalala genotype from Azerbaijan to study how tomato plants (*Solanum lycopersicum*)

respond to both virus and drought stresses. The Shalala seeds came from the Ministry of Agriculture, Horticultural Research Institute of Azerbaijan Republic. Seeds were planted in a special room without insects at a temperature of 26/20°C during the day and night. They were exposed to 16 hours of light and 8 hours of darkness each day, air humidity being 60-70%. After 2-3 weeks, young tomato plants were moved to 2-litre pots. The plants were split into four groups: healthy-H plants (control variant), virus-inoculated plants (V), drought-affected plants (D), and plants affected by both virus and drought (VD). The main highlight lies in the research conducted with 15 tomato plants in each group, a total of 180 tomato plants. The work had been conducted in 3 biological replicates. While conducting the research, we moved whiteflies (*Bemisia tabaci*) from virus-infected tomato plants to healthy tomato plants. Tomato plants were put in boxes made of a special material called polycarbonate that stops insects from getting in. Around 25 whiteflies were put on each tomato plant to feed. 3-4 weeks later, the experimental plants demonstrated signs of TYLCV.

DNA extraction and polymerase chain reaction (PCR): Using the CTAB method, these plants provided leaf samples and total DNA was extracted from them (Aboul-Maaty et al., 2019). Nano Drop1000 (Thermo Scientific) was utilized to analyze DNA concentration and purity spectrophotometrically. Later, using special primers MA13/MA26 (5'-AATGCAATCTTCGT CACC-3'/5'-CGCCCGTCTCGAAGGTTTCG-3'), additional duplicates of the DNA samples were created for TYLCV detection. PCR reaction in 25 µl volume: 1X Taq Buffer; 2 mM MgCl₂; 0.15 mM dNTP mix; 0.2 µM forward and reverse primer; 1.24 U Taq DNA polymerase; and collected with 100 ng of DNA. PCR: pre-denaturation at 95 °C for 1 min. 35 cycles (denaturation at 95 °C for 30 s, annealing at 65 °C for 1 min and elongation at 72 °C for 1 min) and a final elongation at 72°C for 10 min were performed. PCR response items were visualized on a 1% TBE agarose gel utilizing ethidium bromide in a gel documentation gadget (Uvitek, Britain). PCR analysis showed that a piece of DNA about 1.2 kilobases long was synthesized.

Virus-infected tomato samples were separated into two groups to test the way they handle dry conditions: one group had only drought (D), and the other had TYLCV/drought. There was a 25-day period of drought. On the 10th, 17th, and 24th days of the experiment, the plants were given about ~50 ml of water to avoid dying completely.

Isolation of the enzyme extract: Total cell extract was obtained by homogenizing tomato leaves in a medium containing 1 mM EDTA (pH 8.0), 2 mM phenylmethylsulfonyl fluoride (PMSF), 1% PVP, 100 mM Na phosphate buffer (pH 7.8), 0.1% Triton X-100, 2 mM ascorbate. Furthermore, samples were filtered, and centrifuged for 20 min at 15,000×g. The supernatant remaining after the process was used to study peroxidase enzymes.

Activity of peroxidases: The activities of the studied enzymes in leaves of tomato were assessed spectrophotometrically (Ultrospec 3300 PRO, Amersham, USA) at a linear reaction. Three different samples for each treatment were taken and analyzed twice.

Ascorbate peroxidase (APO, EC 1.11.1.11) activity was measured using a modified version of Nakano and Nakano and Asada's (1981) technique. The technique is based on measuring the rate at which ascorbate breaks down hydrogen peroxide to produce water and dehydroascorbate. At 290 nm, optical density was measured in a spectrophotometer. The activity was determined using a molar extinction coefficient of $\epsilon=2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed in $\mu\text{mol}(\text{ascorbate}) \text{ mg}^{-1} (\text{protein}) \text{ min}^{-1}$.

The increase in optical density of the reaction mixture for one minute at 590 nm was used to test

the **activity of the benzidine-type peroxidase (BPO EC 1.11.1.7.)** (Gechev et al., 2002). By taking into account the extinction coefficient, $\epsilon=39 \text{ mM}^{-1} \text{ cm}^{-1}$, the activity was estimated in $\mu\text{mol} (\text{benzidine product}) \text{ mg}^{-1} (\text{protein}) \text{ min}^{-1}$.

Statistical analysis: The unequal variance two-tailed Student's t-test was utilized to examine the significance of variations between the control and experimental groups. P-values less than 0.05 were regarded as statistically significant data. For every treatment, three distinct samples were obtained and subjected to two analyses.

RESULTS AND DISCUSSION

Comparing the leaves of the uninfected control plants to the infected leaf samples, a significant rise in the activities of APO, and BPO was discovered. Every stressed plant manifested a high level of peroxidase activity. Samples from groups V and D exhibited higher BPO and GPO activity, while samples from groups VD showed less activity than V and D groups of plants (Figure 3, 4). Therefore, APO and BPO activity were respectively in control tomatoes 47 and 17 ($\mu\text{mol H}_2\text{O}_2/\text{mg protein} \cdot \text{min}$), Virus infected samples 66 and 23 ($\mu\text{mol H}_2\text{O}_2/\text{mg protein} \cdot \text{min}$), drought-treated plants 70 and 22 ($\mu\text{mol H}_2\text{O}_2/\text{mg protein} \cdot \text{min}$) and dual stress applied tomatoes 50 and 19 ($\mu\text{mol H}_2\text{O}_2/\text{mg protein} \cdot \text{min}$). The current study found that certain viral infections cause significant alterations in the components of plants' antioxidant defense systems.

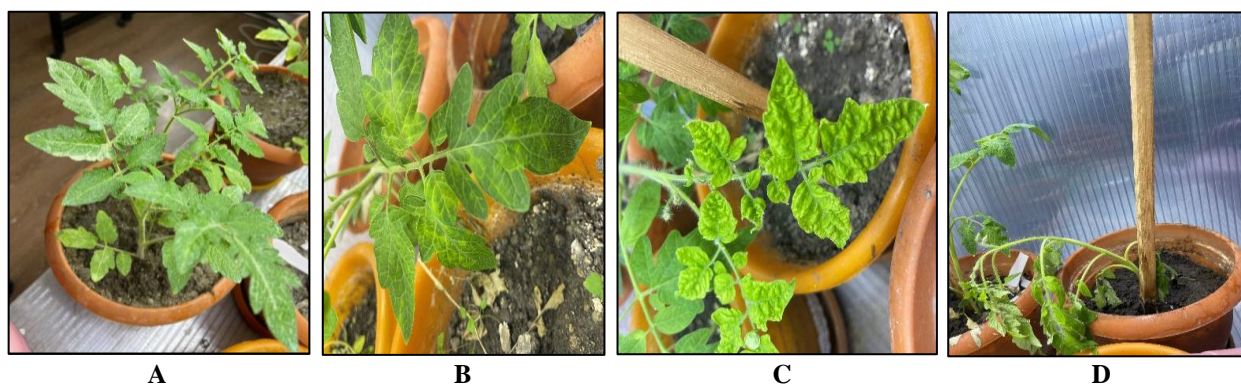


Fig. 1. The tomato plant grown under artificial climatic conditions (8/16 photoperiod, 26/20±1°C temperature, 60–70 % relative humidity): control (A), TYLCV infected (B), drought treatment (C), combined stress (D).

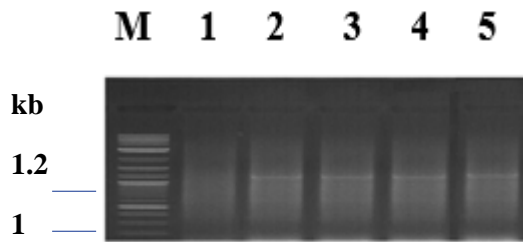


Fig. 2. M-100 bp DNA ladder, 1-negative control; 2; 3; 4, 5 TYLCV infected tomato samples (Shalala).

Tomatoes (*Solanum lycopersicum L.*) are widely cultivated and highly popular vegetables worldwide. Many individuals worldwide enjoy consuming tomatoes because of their delicious flavor and numerous health benefits (Alhudiab et al., 2014; Chen et al., 2016). TYLCV is responsible for causing harm to tomato plants through the development of the tomato yellow leaf curl disease (Michael et al., 2009; EL-DougDoug et al., 2013). This disease, as noted by Lefeuvre et al., (2010) and Deng et al., (2015) is one of the most pernicious in the world. TYLCV-EGS and TYLCV-EGT isolates may both cause symptoms when physically injected into healthy plants using a syringe and then spread by whiteflies (Mirzayeva et al., 2023). Additionally, it has been stated that understanding the cellular components of a virus-infected plant is crucial to comprehending the host cell's actions as well as the type and degree of harm the virus has produced. According to the results of the current investigation, infected tomato plants with TYLCV-EGS and TYLCV-EGT had altered metabolic and biochemical parameters when compared to healthy controls across four distinct tomato cultivars.

Hu et al., (2007) reported an increase in sodium content synchronized with an increase in drought stress in their research on *Purslane* (*Portulaca oleracea L.*) leaves. When examining the effect of drought stress on the ionic contents in soybean shoots, Niakan and Ghorbanli (2007) found out that sodium level increased by stress relative to blank level, but potassium concentration decreased. They state that the decrease in water potential, which in turn led to a decrease in the plant's ability to transfer potassium from the root to the shoot, is the source of the

shoot's decreased potassium concentration. An increase in sodium levels in stressed plants is a defense mechanism that helps the plants regulate cell osmotic pressure and soil solute uptake of water and nutrients.

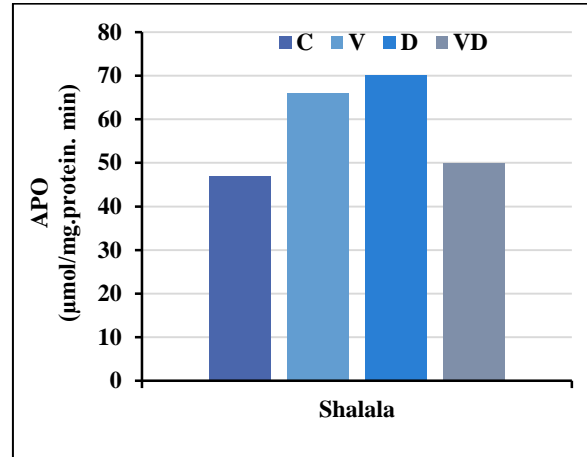


Fig. 3. Ascorbate peroxidase (APO) activity in stressed Shalala genotype ($\mu\text{mol}/\text{mg protein}\cdot\text{min}$): C-control plants, V-TYLCV infected tomatoes, D-drought treatment, VD-virus and drought applied samples (combined stress).

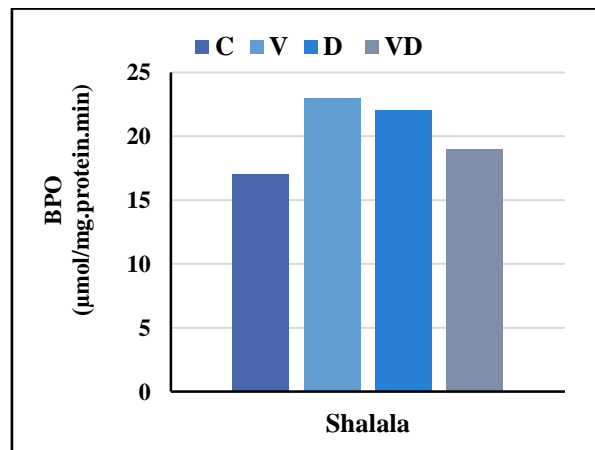


Fig. 4. Benzidine peroxidase (BPO) activity in stressed Shalala genotype ($\mu\text{mol}/\text{mg protein}\cdot\text{min}$): C-control plants, V-TYLCV infected tomatoes, D-drought treatment, VD-virus and drought applied samples (combined stress).

In another study, proline content enhanced in tobacco mosaic virus (TMV)-infected pepper (*Capsicum annuum L.*) plants compared to corresponding controls (Sercan, 2013; Pazarlar et

al., 2013). In order to successfully block off the pathogen and stop the disease process, plants that are exposed to microbial pathogens release reactive oxygen species (ROS) that cause the plant cells surrounding the infection site to undergo programmed cell death (Apel and Hirt, 2004; Moshe et al., 2012). One of the most important effects of different stresses is the creation of oxidative stress, which develops under high levels of reactive oxygen species (ROS) in cells subjected to stress (Kumar et al., 2010). Plants develop complex antioxidant metabolism to improve the damage caused by ROS. The creation of lignin and other oxidative phenols that amplify the cell structure when attacked by pathogens are catalyzed by PPO and POD enzymes (Deng et al., 2015). Studies report that TYLCV-infected tomato plants had higher levels of antioxidants like BPO, PPO, APO, and CAT compared to healthy plants. This was true for all the different groups of tomato plants we involved in experiments. The acquired results happened to overlap with the research done by Sudhakar et al. (2006), Rai et al. (2011), Jaiswal et al. (2012), Huseynova and Aliyev (2012) and Sofy et al. (2013). According to these publications, activities of leaf antioxidant enzymes saw an increase when affected by biotic stress.

In all the plants tested, both amylase and protease enzymes led to a significant increase in TYLCV-infected samples (Sofy et al., 2017). TYLCV-EGT isolate demonstrated the most essential impact on both antioxidant and hydrolytic enzyme activities out of the two types of TYLCV isolates. Antioxidant enzymes are activated as a result of the stress in infected tomato cultivars. Plants have evolved advanced antioxidant systems to shield cellular membranes and organelles from the destructive effects of ROS (Šubr et al., 2006; Sofy et al., 2014). Viral infection causes an increase in peroxidase activity, which was observed in peaches, apricots (Diaz-Vivancos et al., 2006; Radwan et al., 2010).

Peroxides appeared to be pivotal pathogenesis-related proteins (PR-proteins) in the works of Almagro et al. (2009) and Sofy et al. (2014). They play a crucial role in defending plants against pathogens, by participating in the deletion process of hydrogen peroxide from the cells. Thus, the timing and location of higher

levels of guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) activity, and their role in the stiffening of cell walls, show that peroxidases help create substances that block pathogen penetration. It was reported that infected plant parts had higher levels of SOD, CAT, APO, and BPO activities and hydrogen peroxide when they were infected with a virus. An increase in viral infection prompted the heightened activity of SOD, CAT, GPX, and APX, demonstrating their roles in eliminating harmful substances from ROS.

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