UDC 634.8:615.9

ENDOGENOUS ANTIOXIDANT DEFENSE RESPONSES IN GRAPEVINE PLANTS TO THE INFECTION OF GLRAV-3

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Received October, 15, 2025; revised November, 5, 2025; accepted April, 22, 2026

Aim. The aim of this study was to investigate how viral infections, which severely impair plant growth and productivity, affect reactive oxygen species (ROS) accumulation, and to evaluate the roles of both enzymatic antioxidant defenses and non-enzymatic low–molecular weight antioxidants, including ascorbate (ASA), glutathione (-L-glutamyl-L-cysteinyl-glycine = GSH), and tocopherols (TOCs), in mitigating virus-induced oxidative stress in plants. **Metods.** In the present study, a total of 45 leaf samples were collected from mature 10-year-old vines of the cultivars 'Gara Shani' (red) and 'Ag Shani' (white). Sampling was conducted in three vineyards, where five vines were selected per vineyard, and three physiologically mature leaves were collected from each vine, resulting in 15 leaves per vineyard and 45 leaves in total (3 vineyards × 5 vines × 3 leaves). In each vineyard, one vine without visible symptoms was designated as a healthy control, while the remaining vines exhibited typical viral symptoms such as reddening of leaf margins and petioles, reduced vigor, leaf rolling, yellowing, and curling. Consequently, 36 leaves were obtained from symptomatic vines and 9 leaves from asymptomatic (healthy) vines. Grapevine leafroll-associated virus 3 (GLRaV-3) infection was confirmed in grapevine samples using AgriStrip rapid assay, double-antibody sandwich ELISA (DAS-ELISA), and reverse transcription–polymerase chain reaction (RT-PCR). Subsequently, virus-induced changes in non-enzymatic antioxidants were quantified by spectrophotometric determination of ascorbate and glutathione, tocopherols via the Emmerie–Engel reaction, and lipid peroxidation through MDA content using the thiobarbituric acid reactive substances (TBARS) assay. Enzymatic antioxidant activities, including ascorbate peroxidase and catalase, were measured spectrophotometrically, and soluble protein content was determined by the Sedmak method. **Results.** Viral screening using AgriStrip assays and DAS-ELISA, followed by RT-PCR targeting the coat protein gene, confirmed GLRaV-3 infection in 13 samples (approximately 36,1%), while symptomless vines tested negative, demonstrating consistency between visual assessment and molecular diagnostics. GLRaV-3 infection resulted in significant increases in non-enzymatic antioxidant levels compared with healthy controls. Tocopherol (TOC) content increased by 18–73% across infected samples 2–6, whereas sample 1 showed no significant change. Glutathione (GSH) levels were elevated by 20–60% in infected samples 1, 2, 4, 5, and 6, while sample 3 showing no significant difference. Ascorbic acid (ASA) content increased by 10–23% in infected samples 1, 3, 5, and 6, with sample 2 and 4 showing no significant difference. Lipid peroxidation, as indicated by malondialdehyde (MDA) content, was also significantly enhanced in infected leaves. MDA levels increased approximately 1.5–2.6-fold in samples 1, 2, 4, 5, and 6 compared with healthy controls, reaching absolute values of 0.85–1.75 μmol g^{-1} FW versus 0.45–0.9 μmol g^{-1} FW in controls. In parallel, the activities of key antioxidant enzymes were elevated: catalase (CAT) activity increased by 25–48%, and ascorbate peroxidase (APX) activity increased by 30–55% in infected leaves compared with corresponding controls. **Conclusions.** Surveys conducted in Salyan vineyards confirmed GLRaV-3 infection supporting the link between observed symptoms and biochemical oxidative stress responses. These biochemical changes demonstrate that GLRaV-3 infection triggers oxidative stress and concurrently activates both enzymatic and non-enzymatic antioxidant defense mechanisms in grapevine leaves. The coordinated increase in ASA, GSH, TOC, CAT, and APX, alongside elevated MDA, indicates a complex redox adjustment aimed at mitigating virus-induced oxidative damage.

Keywords: grapevine, viruses, metabolites, antioxidant enzymes.

DOI: https://doi.org/10.15407/agrisp13.01.050

INTRODUCTION

Viral diseases remain a major constraint to sustainable grapevine production worldwide. Among them, grapevine leafroll disease (GLD) represents one of the most economically significant viral diseases affecting viticulture (Bertami et al., 2005; Gilardi et al., 2020). GLD is associated with several species collectively referred to as Grapevine leafroll-associated viruses (GLRaVs), which belong to the family *Closteroviridae* (Maree et al., 2013). These viruses induce characteristic symptoms including interveinal reddening or yellowing, downward leaf rolling, delayed fruit ripening, and reduced sugar accumulation, ultimately leading to significant yield and quality losses (El Aou-Ouad et al., 2016). Within this complex, Grapevine leafroll-associated virus 3 (GLRaV-3) is classified in the genus Ampelovirus and is widely recognized as the predominant and most economically important causal agent of GLD. GLRaV-3 is usually unevenly distributed in infected vines and establishes long-term systemic infections typical of perennial woody hosts such as *Vitis vinifera* (Montero et al., 2016).

Presently, there exists a substantial amount of information regarding the interactions between plants and viruses, as well as the effects of viral infections on the plant life cycle in annual plants (Garcia et al., 2022). However, the effects of viral infections in perennial plants are notably scarce (Takahashi et al., 2019). Transmission of GLRaV-3 occurs primarily through vegetative propagation and grafting, as well as by phloem-feeding insect vectors, particularly soft scale insects (*Coccidae*) and mealybugs (*Pseudococcidae*) (Maree et al., 2013). The epidemiology of GLD is therefore strongly influenced by vineyard management practices and vector population dynamics and control.

Unlike annual plants, grapevine is a perennial crop that remains infected for decades once virus infection is established. Consequently, virus–host interactions in grapevine involve long-term physiological and metabolic adjustments, which may vary across developmental stages and growing seasons (Bertamini et al., 2004; Sharma et al., 2024). Despite the economic importance of GLD, the biochemical responses of grapevine to chronic GLRaV-3 infection remain incompletely characterized, particularly under field conditions (Sultanova et al., 2019; Sultanova et al., 2024; Sultanova et al., 2025). The expression of GLD symptoms depends on factors such as season, grape cultivar, and regional climatic conditions. Some cultivars, particularly certain white *Vitis vinifera* varieties and rootstocks, may remain asymptomatic, resulting in latent infections (Cui et al., 2016). Initial leaf symptoms generally appear in early to midsummer and persist until late autumn, progressively intensifying. GLD not only affects visual traits but also significantly reduces grape yield and quality, highlighting the need for effective vineyard management strategies.

Viral infection triggers the production of reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$), which can disrupt cellular functions and activate plant defense mechanisms (Xu et al., 2024). An imbalance between ROS and antioxidants results in oxidative stress, damaging proteins, lipids, and nucleic acids (Xu et al., 2024). Viral stress can further alter biochemical and physiological processes, affecting photosynthesis, protein synthesis, and solute accumulation (Carvalho et al., 2015). The interplay between viral infection and ROS signaling is complex, influencing the ability of the host to maintain cellular integrity while activating defense responses. Our previous studies have shown that the adverse effects of viral stress led to a significant reduction in green pigments like chlorophylls (*a*, *b*, and total) and a gradual reduction in carotenoids, as well as the level of relative water content (RWC) and the increased amount of hydrogen peroxide in all infected leaves (Bayramova et al., 2018 and 2021).

Although the physiological and biochemical consequences of viral infections in plants have been extensively documented, the contribution of endogenous non-enzymatic antioxidants — particularly the ascorbate–glutathione–tocopherol triad — to virus tolerance remains insufficiently explored (Ramzan et al., 2021). Existing studies have primarily focused on individual antioxidants or enzymatic responses, while the coordinated behavior of this antioxidant triad under viral stress conditions has received comparatively little attention (Kuzniak et al., 2017; Singh et al., 2024). Nonetheless, recent research efforts worldwide have begun to investigate the involvement of the ascorbate–glutathione–tocopherol system in enhancing plant resistance to diverse biotic stresses, including bacterial and fungal pathogens (Singh et al., 2024).

Ascorbic acid (ASA), commonly referred to as vitamin C, is a key non-enzymatic antioxidant that

mitigates oxidative stress by scavenging reactive oxygen species produced under biotic stress, including viral infections (Hancevic et al., 2022). Glutathione (GSH) is a low-molecular-weight tripeptide thiol that is ubiquitously distributed across multiple cellular compartments, including the cytosol, mitochondria, chloroplasts, peroxisomes, vacuoles, and the endoplasmic reticulum (Otulak-Kozieł et al., 2022). It plays a central role in maintaining cellular redox homeostasis, regulating cell death and differentiation, modulating enzyme activity, and mediating responses to biotic stress, including pathogen attack (Clarke et al., 2002). The ascorbate–glutathione–tocopherol triad operates as an integrated redox network, providing comprehensive cellular protection against oxidative stress (Szarka et al., 2012). Ascorbate reduces oxidized tocopherol, thereby sustaining its membrane-protective function; glutathione, in turn, regenerates ascorbate and supports redox cycling (Faizan et al., 2023). Tocopherols play a crucial role in safeguarding membrane lipids from peroxidative damage, while ascorbate and glutathione contribute to redox signaling, metabolic regulation, and stress adaptation (Faizan et al., 2023). Together, these metabolites form a dynamic antioxidant system that not only detoxifies ROS but also participates in broader regulatory processes, including the modulation of signaling pathways and secondary metabolite biosynthesis (Szarka et al., 2012). GLRaV-3 is a widely distributed virus causing significant yield and quality losses in grapevine (*Vitis vinifera*) worldwide, including in Azerbaijan (Sultanova et al., 2024). This study evaluated the oxidative stress responses of grapevine leaves under natural GLRaV-3 infection, focusing on symptom expression, reactive oxygen species (ROS) accumulation, and antioxidant system modulation (Bayramova et al., 2018, 2021; Sultanova et al., 2025). We hypothesized in the present study that GLRaV-3 infection triggers oxidative stress, reflected in changes in ROS levels and antioxidant activities. End-point concentrations of the ascorbate–glutathione–tocopherol (ASC–GSH–TOC) triad were assessed, alongside the activities of key antioxidant enzymes, catalase (CAT) and ascorbate peroxidase (APX). To this end, with a limited set of local Azerbaijani grapevine samples, it was aimed to provide preliminary insights into the potential role of endogenous antioxidants in mitigating virus-induced oxidative stress. This could contribute to a better understanding of host defense mechanisms and the physiological impact of GLRaV-3 in viticulture.

MATERIALS AND METHODS

Plant material. Surveys were conducted during 2023 in Salyan, one of the major grapevine-growing regions of Azerbaijan, to monitor grapevine leafroll disease (GLD) symptoms. A total of 45 leaf samples were collected from mature 10-year-old vines of the cultivars ‘Gara Shani’ (red) and ‘Ag Shani’ (white) located in three vineyards. From each vineyard, five vines were selected and three fully expanded and physiologically mature leaves were collected per vine, resulting in 15 leaves per vineyard and 45 leaves in total. Among the sampled vines, 36 leaves were obtained from symptomatic (infected) vines, while 9 leaves were collected from asymptomatic vines, each serving as a healthy control corresponding to one of the infected vines, exhibiting typical viral symptoms such as reddening of leaf margins and petioles, reduced vigor, leaf rolling, yellowing, and curling **(Fig. 1)**. Sampling was performed between 09:00 and 11:00 a.m. under sunny conditions with ambient temperatures of 20–25°C to minimize diurnal variation in antioxidant and metabolite levels. Meteorological data (monthly maximum and minimum air temperature (°C), relative humidity (%), and rainfall (mm)) were obtained from the Salyan regional meteorological station of the Azerbaijan National Hydrometeorological Service and used to characterize seasonal temperature dynamics in the study area **(Fig. 2)**.

Accordingly, from each vine, three fully expanded and physiologically mature leaves were collected, and five vines per vineyard site were sampled to constitute one composite experimental sample. In total, samples were obtained from three productive vineyards managed under standard agronomic practices. Immediately after harvesting, leaf samples were transported to the laboratory under cooled conditions and stored at 4°C. All biochemical analyses were completed within 24 h of sampling to prevent degradation of ascorbate (ASA), glutathione (GSH), and tocopherol (TOC). The experimental procedures were conducted at the Laboratory of Bioadaptation, Institute of Molecular Biology and Biotechnologies, Ministry of Science and Education of the Republic of Azerbaijan. All analyses were performed by the author under controlled laboratory conditions. Healthy control samples were collected from virus-free vines of the same cultivars located in the same or neighboring vineyards to minimize potential confounding effects associated with (hidden) variables of cultivar type, vine age, and local environmental factors. Only samples with labo-

Fig. 1. Typical foliar symptoms observed on white (upper panels, 'Ag Shani') and red (lower panels, 'Gara Shani') grapevine cultivars infected with Grapevine leafroll-associated virus 3 (GLRaV-3). In white cultivars, infected leaves exhibit interveinal chlorosis, downward rolling of leaf margins, and reduced vigor compared with healthy plants. In red cultivars, characteristic symptoms include interveinal reddening while the primary and secondary veins remain green, accompanied by leaf rolling and lamina thickening. Symptom expression was recorded under field conditions during the vegetative growth stage. In total, 45 grapevine samples were collected (15 from 'Ag Shani' and 30 from 'Gara Shani'), of which 13 tested positive for GLRaV-3 (5 from 'Ag Shani' and 8 from 'Gara Shani'). Nine asymptomatic leaves served as healthy controls, each corresponding to one of the infected vines

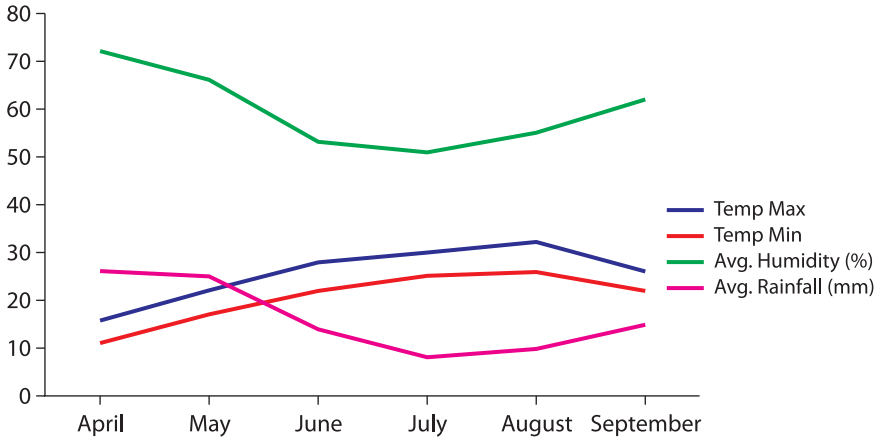

Fig. 2. Monthly maximum (Max T) and minimum (Min T) air temperatures (°C), relative humidity (%), and rainfall (mm) in the southwest of Salyan (Azerbaijan) during the grapevine growing season for the period April through September, representing typical climatic conditions in the study region

ratory-confirmed (see below) infection by Grapevine leafroll-associated virus 3 (GLRaV-3) were included in the biochemical analyses.

Virus detection and identification. Collected leaf samples were analyzed for the presence of GLRaV-3 using a combination of rapid one-step AgriStrip assay (Bioreba AG, Reinach, Switzerland), DAS-ELISA, and RT-PCR. AgriStrip assay: Plant extracts were incubated with magnetic beads coated with antibodies specific to GLRaV-3, following the manufacturer's instructions (Bioreba AG, Reinach, Switzerland). The appearance of a colored test line indicated a positive reaction. DAS-ELISA: The assay was performed according to the methodology described by Clark and Adams (1977) and Zimmermann et al. (1990), using commercial ELISA kits (Bioreba AG, Reinach, Switzerland; Agdia, Inc., Elkhart, IN, USA). The tissue/extraction buffer ratio was 1:20 (w/v) for Bioreba kits and 1:10 (w/v) for Agdia kits. Absorbance at 405 nm was measured at least twice using a Stat Fax Microplate Reader (Awareness Technology, USA). Positive and negative controls supplied with the commercial Bioreba GLRaV-3 diagnostic kits (Bioreba AG, Reinach, Switzerland) were included in all Agri Strip and DAS-ELISA assays according to the manufacturer's instructions to ensure the reliability and accuracy of the results. RT-PCR: Total RNA was extracted from 200 mg of leaf tissue using Trizol reagent (Invitrogen, USA) and stored at –80°C. RNA quality and concentration were determined using a NanoDrop spectrophotometer (Thermo Scientific, USA). cDNA synthesis and PCR amplification were performed using virus-specific primers LR3-9445c (5′-CTACTTCTTTTGCAATAGTT-3′) and LR3-8504v (5′-ATGGCATTTGAACTGAAATT-3′), originally designed by Ling et al. (2004), following the method of Fajardo et al. (2007) with a two-step RT-PCR protocol. PCR products were resolved on 1.5% agarose gels in 1X TBE buffer, stained with ethidium bromide, and visualized under UV light using a UV-Gel Doc system (UK). A 2-log DNA ladder (NEB, UK) was used to approximate the molecular weight of amplified products. For RT-PCR assays, RNA extracted from the GLRaV-3 positive control supplied with the Bioreba diagnostic kit (Bioreba AG, Reinach, Switzerland) was used as the positive control to validate the assay. RNA extracted from healthy grapevine leaves and nuclease-free water (no-template control) were included as negative controls to verify the absence of contamination and non-specific amplification in all reactions.

Tocopherol determination. Tocopherol was estimated in plant samples using 0.5 g of fresh leaf tissue according to the Emmerie–Engel reaction (Emmerie and Engel, 1938). It is based on the reduction of ferric to ferrous ions by tocopherols, which, with 2, 2′-dipyridyl, forms a red color. Six GLRaV-3–infected samples were analyzed: samples 1–3 corresponded to the white cultivar 'Ag Shani', whereas samples 4–6 corresponded to the red cultivar 'Qara Shani'. Healthy control samples were collected from virus-free vines of the same cultivars located in the same or neighboring vineyards to minimize environmental variation. Each infected sample was analyzed and compared with its corresponding cultivar-specific healthy control. Tocopherols and carotenes were first

extracted with xylene and read at 460 nm to measure carotenes using a Thermo Scientific Evolution 350 UV/Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). A correction was made for this after adding ferric chloride and reading at 520 nm. α-Tocopherol was used as the standard in accordance with the Emmerie–Engel method. The concentration of tocopherol in the sample was calculated using this formula:

$$\text{Tocopherols (µg/mg fresh weight, FW)} = \\ = ((\text{Sample A520} - \text{A460}) / \text{Standard A520}) \times \\ \times 0.29 \times 0.15$$

where 0.29 represents the extinction coefficient–based conversion factor and 0.15 accounts for dilution and extraction volume.

Glutathione determination. Approximately 0.5 g of fresh leaf tissue was used for each assay. GSH content was measured spectrophotometrically at 412 nm using a glutathione reagent kit (Sigma-Aldrich, Cat. No. CS0260) following the manufacturer's protocol. The reaction mixture contained 95 mM potassium phosphate buffer (pH 7.0), 0.95 mM EDTA, 0.038 mg/ml (48 µM) NADPH, 0.031 mg/ml DTNB, 0.115 units/ml glutathione reductase, and 0.24% 5-sulfosalicylic acid. A calibration curve was prepared using standard GSH solutions (0.5–20 µg/ml) in 0.4 M Tris-HCl buffer (pH 8.5) according to the kit instructions (Sigma-Aldrich).

Determination of lipid peroxidation. The intensity of the lipid peroxidation process in plants was calculated in terms of MDA in healthy and infected leaf samples. This method primarily quantifies free cytosolic malondialdehyde (MDA), commonly used as an indicator of lipid peroxidation in plant tissues. The MDA content was determined spectrophotometrically using a Thermo Scientific Evolution 350 UV/Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) through its reaction with 0.5% thiobarbituric acid at wavelengths 532 nm and 600 nm. The formula used for calculation was A = (D532 − – D600)/(E × m), where A represents MDA concentration, D is the optical density, m denotes plant wet biomass, and E is a constant coefficient (Knight et al., 1988).

L-Ascorbic acid (ASA) determination. In an acidic environment, ASA reduces Fe^{3+} to Fe^{2+} and forms iron hexacyanoferrate. To determine the amount of ascorbic acid, 0.5 g of leaves were crushed in a solution containing 0.1 M Na-citrate (pH 3.69) and collected in tubes. In the next process, it was centrifuged at 12,500 g for 5 minutes and the supernatant was collected. A mixture of 25 µl of 1% potassium ferricyanide $K_3[Fe(CN)_6]$ and 25 µl of 2% NaFe was added to 500 µl of the supernatant and kept at room temperature for 5 minutes. 50 µl of 2% $FeCl_3$ was added to 1.9 ml of distilled water, and incubated for 5 minutes, and the optical density at 680 nm wavelength was determined (Thermo Scientific Evolution 350 UV-Vis Spectrophotometer, England). The amount of ascorbic acid was calculated using the following formula:

$$C = (K \times V \times X) / (m \times \Delta m \times L),$$

where C — the amount of ascorbic acid in 1 g of wet biomass (µg); K — concentration of ascorbic acid (µg/ml); V — total volume (ml); X — dilution; L — optical density; m — wet biomass; Δm — the variable represents the coefficient relating dry biomass to wet biomass (Tayar et al., 2022).

Assay of Enzymes Activity. Approximately 0.5 g of fresh leaf tissue was ground in liquid nitrogen and homogenized in 100 mM sodium phosphate buffer (pH 7.8) containing 1 mM EDTA, 2 mM phenylmethylsulfonyl fluoride (PMSF) as a protease inhibitor, 1% polyvinylpyrrolidone (PVP), 0.1% Triton X-100 at 4°C and PMSF was used at a final concentration of 2 mM as a protease inhibitor. Homogenates were centrifuged and the supernatant was used to analyze ascorbate-peroxidase enzyme activity.

Ascorbate peroxidase (APX) activity was determined following Nakano and Asada (1981) with minor modifications: the decrease in absorbance at 290 nm due to ascorbate oxidation was recorded, using 330 nm as the reference wavelength to correct for baseline absorbance, and non-enzymatic oxidation of ascorbate was subtracted by including a control without enzyme extract. APX activity was calculated using the extinction coefficient $\varepsilon = 2.8\ mM^{-1}cm^{-1}$.

Catalase enzyme activity was measured in a spectrophotometer at a wavelength of 240 nm. The measurement was calculated based on the rate of decomposition of H_2O_2 in 1 minute. The reaction composition consisted of 885 µl of 50 mM Na-phosphate (pH 7.8), 25 µl of enzyme extract, and 90 µl of 3% H_2O_2. It was expressed in units of µmol H_2O_2/mg protein min, taking $\varepsilon = 39.5\ mM^{-1}cm^{-1}$ as the molar extinction coefficient (Kumar & Knowles, 1993). Soluble protein content was determined at 595 nm, with bovine

serum albumin used to construct the calibration curve (Sedmak & Grossberg, 1977).

Statistical analyzes. All statistical analyses were performed using Student's t-test implemented in IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Experiments were conducted using three independent biological replicates per group ($n = 3$). Data are expressed as mean ± standard error (SE). Differences between GLRaV-3-infected vines and their corresponding healthy controls were considered statistically significant at $P < 0.05$. To further investigate the relationships among oxidative stress markers and antioxidant defense components, Pearson correlation analysis was performed including both non-enzymatic antioxidants (ascorbic acid, ASA; glutathione, GSH; tocopherol, TOC), lipid peroxidation marker (malondialdehyde, MDA), and antioxidant enzymes (catalase, CAT; ascorbate peroxidase, APX). The resulting correlation matrix was visualized as a heatmap to illustrate the strength and direction of pairwise associations among all measured biochemical parameters. Correlation coefficients (r values) were calculated based on biological replicates, and the heatmap enabled comprehensive evaluation of coordinated enzymatic and non-enzymatic antioxidant responses under GLRaV-3 infection.

RESULTS

During the 2023 growing season, systematic surveys were conducted in vineyards to assess viral infections in grapevines. Both red and white grapevine cultivars were included in the study **(Fig. 1)**. In April, the average maximum and minimum air temperatures were 16°C and 11°C, respectively, reflecting mild spring conditions at the onset of active vegetative growth. Temperatures gradually increased toward mid-summer, reaching peak values in August, when the average maximum temperature was 32°C and the minimum was 26°C. These elevated summer temperatures represent typical climatic conditions for the Salyan region and may contribute to enhanced physiological and oxidative stress responses in grapevine plants infected with GLD. In addition to temperature, available climate data indicate that the region has a semi-arid climate with relatively low rainfall during summer months (e.g., June–August receive minimal precipitation) and moderate relative humidity, which may influence vine physiology (humidity usually ranges from ~50% in summer to ~70% in spring–autumn; average precipitation in summer months is low with a pronounced dry period). Temperature, humidity, and precipitation data reflect typical climatic conditions in the study region and may influence vine physiology, vector activity (e.g., mealybugs), and virus spread. The monthly variation in maximum and minimum temperatures (°C), relative humidity (%), and rainfall (mm) from April to September 2023 is presented in **Fig. 2**.

Viral infection was initially screened using the rapid one-step AgriStrip assay and DAS-ELISA. Among the 36 symptomatic grapevine samples (12 from 'Ag Shani' and 24 from 'Gara Shani'), 13 tested positive for Grapevine leafroll-associated virus 3 (GLRaV-3) (5 from 'Ag Shani' and 8 from 'Gara Shani'). RT-PCR confirmed GLRaV-3 infection in the same 13 samples, corresponding to an overall detection frequency of approximately 36% (13/36) among symptomatic samples, whereas no amplification was detected in healthy control leaf extracts. The remaining 9 asymptomatic (healthy) leaves, along with the 23 symptomatic but virus-negative leaves, tested negative by both DAS-ELISA and RT-PCR, supporting the consistency between visual assessment and laboratory confirmation within the examined material.

Following laboratory confirmation of GLRaV-3 infection in 13 samples, three infected grapevine plants from a white cultivar ('Ag Shani') and three from a red cultivar ('Qara Shani') were randomly selected for subsequent biochemical analyses. In each vineyard, five vines were initially surveyed, of which one vine without visible symptoms and testing negative for GLRaV-3 was designated as the healthy control. For each infected plant, a corresponding healthy control vine of the same cultivar and comparable age was selected from the same or a neighboring vineyard. In total, three infected and three healthy control biological replicates were included in the study ($n = 3$ per group). All biochemical measurements were performed using these independent biological replicates, with each analysis conducted in triplicate technical repetitions. Virus detection was based on qualitative confirmation by DAS-ELISA and RT-PCR. Viral titre was not quantified; therefore, analyses were performed according to infection status (infected vs. healthy) rather than viral load.

GLRaV-3 infection induced a consistent increase in tocopherol (TOC) content across both grapevine cultivars **(Fig. 3)**. In the white cultivar 'Ag Shani' (samples 1–3), TOC increased from approximately

0.85 to 0.95 mg g^{-1} fresh weight (FW) in sample 1 (≈12% increase, non-significant, ns), from 0.92 to 1.15 mg g^{-1} FW in sample 2 (≈25% increase;, $P<0.01$), and from 0.78 to 0.97 mg g^{-1} FW in sample 3 (≈24% increase;, $P<0.01$). In the red cultivar 'Qara Shani' (samples 4–6), the increase was more pronounced, rising from 0.66 to 0.99 mg g^{-1} FW in sample 4 (≈50% increase;, $P<0.001$), from 0.73 to 0.98 mg g^{-1} FW in sample 5 (≈34% increase;, $P<0.001$), and from 0.88 to 1.05 mg g^{-1} FW in sample 6 (≈19% increase, $P<0.01$). These results indicate a marked enhancement of lipid-soluble antioxidant capacity under viral stress, particularly in the red cultivar.

Glutathione (GSH) levels also increased in infected plants relative to healthy controls **(Fig. 3)**. In 'Ag Shani', GSH rose from approximately 0.25 to 0.33 µmol g^{-1} FW in sample 1 (≈32% increase; ~1.32-fold, $P<0.01$) and from 0.33 to 0.44 µmol g^{-1} FW in sample 2 (≈33% increase; ~1.33-fold, $P<0.01$), whereas sample 3 showed a smaller and statistically non-significant increase from 0.29 to 0.36 µmol g^{-1} FW (≈24% increase, ns). In 'Qara Shani', GSH increased from 0.34 to 0.53 µmol g^{-1} FW in sample 4 (≈56% increase; ~1.56-fold, $P<0.001$), from 0.42 to 0.63 µmol g^{-1} FW in sample 5 (≈50% increase; ~1.50-fold, $P<0.001$), and from 0.29 to 0.45 µmol g^{-1} FW in sample 6 (≈55% increase; ~1.55-fold, $P<0.001$). The magnitude of increase, particularly in the red cultivar, supports an active upregulation of the glutathione-dependent redox buffering system during GLRaV-3 infection.

Ascorbic acid (ASA) content exhibited cultivar- and sample-dependent responses **(Fig.3)**. In 'Ag Shani', ASA increased from 18 to 22 µmol g^{-1} FW in sample

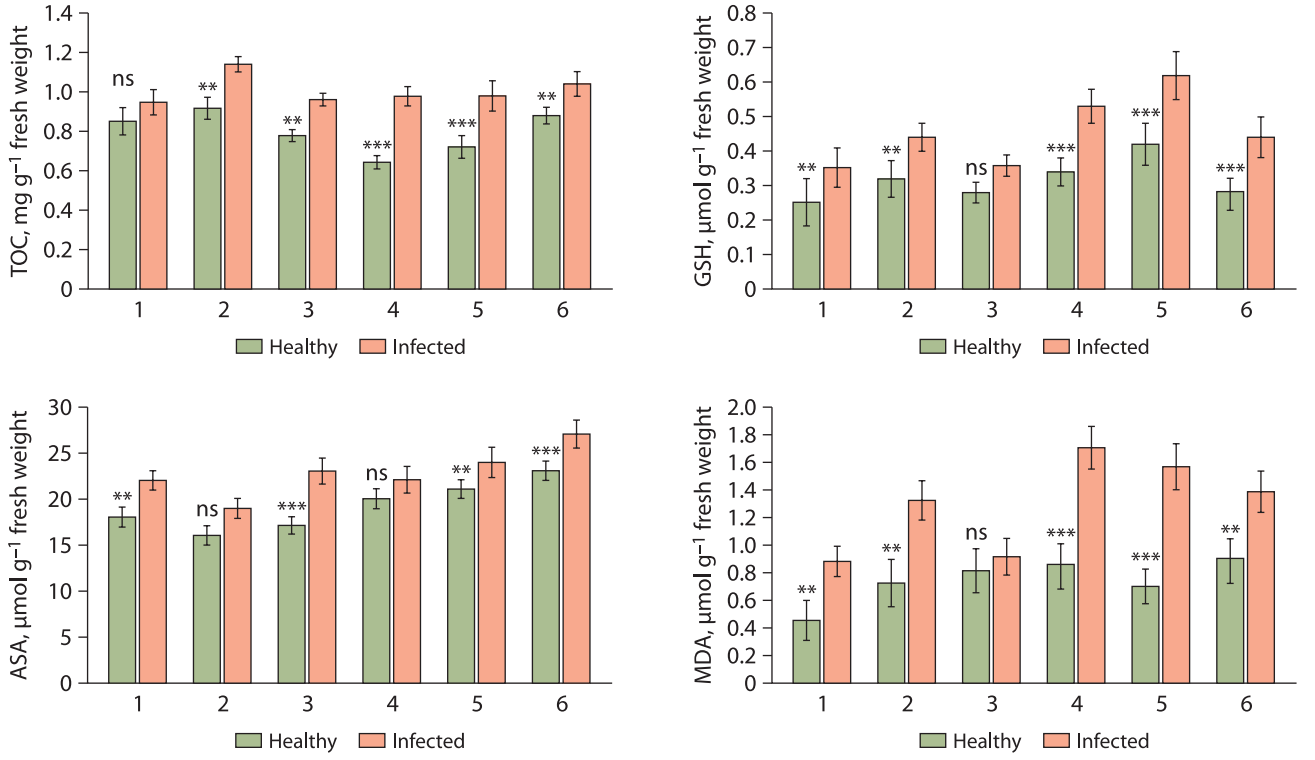

Fig. 3. Changes in non-enzymatic antioxidants and lipid peroxidation in grapevine leaves infected with Grapevine leafroll-associated virus 3 (GLRaV-3). Tocopherol (TOC; mg g^{-1} fresh weight), glutathione (GSH; µmol g^{-1} fresh weight), ascorbic acid (ASA; µmol g^{-1} fresh weight), and malondialdehyde (MDA; µmol g^{-1} fresh weight) contents were determined in healthy control plants and GLRaV-3–infected grapevine samples during the vegetative growth stage. Samples 1–3 correspond to the white cultivar 'Ag Shani', and samples 4–6 correspond to the red cultivar 'Qara Shani'. Data are presented as mean ± SE of three independent biological replicates ($n=3$). Each biological replicate represents an individual grapevine plant randomly selected from laboratory-confirmed GLRaV-3–infected vines, with a corresponding healthy control of the same cultivar and comparable age. All biochemical measurements were performed in technical triplicate for each biological replicate. Asterisks indicate statistically significant differences between infected and corresponding healthy control plants (*$P<0.05$, **$P<0.01$, ***$P<0.001$), whereas ns denotes no significant difference

1 (≈22% increase; ~1.22-fold, $P < 0.01$), showed no significant change in sample 2 (≈16 to 19 µmol g^{-1} FW; ≈19% increase, ns), and increased from 17 to 23 µmol g^{-1} FW in sample 3 (≈35% increase; ~1.35-fold, $P < 0.001$). In 'Qara Shani', ASA rose from 20 to 22 µmol g^{-1} FW in sample 4 (≈10% increase, ns), from 21 to 24 µmol g^{-1} FW in sample 5 (≈14% increase; $P < 0.01$), and from 23 to 27 µmol g^{-1} FW in sample 6 (≈17% increase; ~1.17-fold, $P < 0.001$). Compared with GSH, the ASA response was more moderate and variable, suggesting differential regulation within the ascorbate–glutathione cycle under viral stress.

Malondialdehyde (MDA), a marker of lipid peroxidation, increased substantially in infected plants **(Fig. 3)**. In 'Ag Shani', MDA rose from 0.45 to 0.88 µmol g^{-1} FW in sample 1 (≈95% increase; ~1.95-fold, $P < 0.01$) and from 0.72 to 1.32 µmol g^{-1} FW in sample 2 (≈83% increase; ~1.83-fold, $P < 0.01$), while sample 3 exhibited only a minor, non-significant increase from 0.80 to 0.90 µmol g^{-1} FW (≈12%, ns). In 'Qara Shani', MDA increased from 0.85 to 1.75 µmol g^{-1} FW in sample 4 (≈106% increase; ~2.06-fold, $P < 0.001$), from 0.70 to 1.55 µmol g^{-1} FW in sample 5 (≈121% increase; ~2.21-fold, $P < 0.001$), and from 0.92 to 1.38 µmol g^{-1} FW in sample 6 (≈50% increase; ~1.50-fold, $P < 0.01$). The pronounced elevation of MDA, particularly in the red cultivar, indicates substantial oxidative membrane damage despite the concurrent upregulation of antioxidant systems.

Collectively, the data demonstrate that GLRaV-3 infection triggers a coordinated yet quantitatively stronger increase in lipid peroxidation relative to non-enzymatic antioxidant accumulation, suggesting that antioxidant activation represents a compensatory response that only partially offsets oxidative injury under viral stress conditions.

GLRaV-3 infection resulted in a marked increase in catalase (CAT) activity in both grapevine cultivars **(Fig. 4)**. In the white cultivar 'Ag Shani' (samples 1–3), CAT activity increased from approximately 0.44 to 0.61 µmol mg^{-1} protein min^{-1} in sample 1 (≈39% increase;$P < 0.01$), from 0.56 to 0.73 µmol mg^{-1} protein min^{-1} in sample 2 (≈30% increase; $P < 0.01$), and from 0.35 to 0.58 µmol mg^{-1} protein min^{-1} in sample 3 (≈66% increase;, $P < 0.001$). In the red cultivar 'Qara Shani' (samples 4–6), CAT activity rose from 0.39 to 0.62 µmol mg^{-1} protein min^{-1} in sample 4 (≈59% increase; $P < 0.001$), from 0.48 to 0.74 µmol mg^{-1} protein min^{-1} in sample 5 (≈54% increase, $P < 0.001$), and from 0.52 to 0.88 µmol mg^{-1} protein min^{-1} in sample 6 (≈69% increase; $P < 0.001$). The strongest induction was observed in samples 3 and 6, indicating a substantial enhancement of H_2O_2-scavenging capacity under viral stress, particularly in the red cultivar.

A similar but even more pronounced trend was observed for ascorbate peroxidase (APX) activity **(Fig. 4)**. In 'Ag Shani', APX increased from 0.34 to 0.51 µmol mg^{-1} protein min^{-1} (≈50%

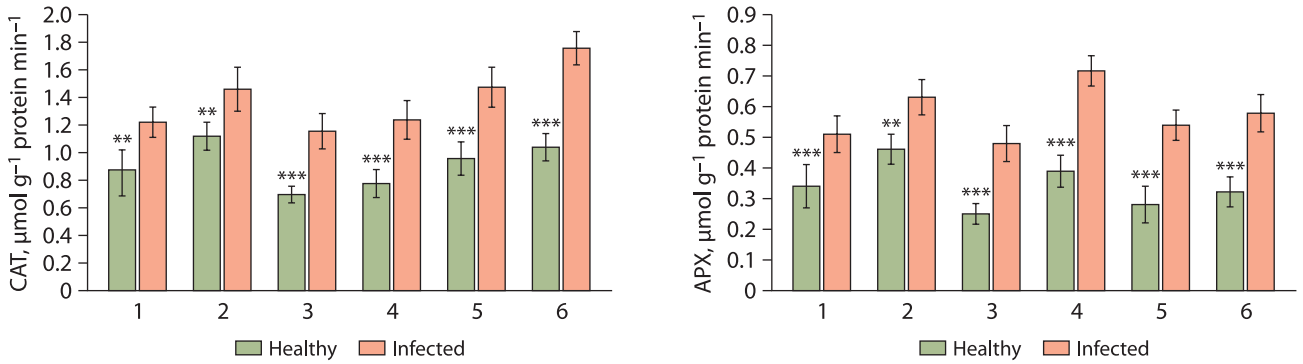

Fig. 4. Activities of ascorbate peroxidase (APX) and catalase (CAT) (µmol mg^{-1} protein min^{-1}) in healthy control plants and GLRaV-3–infected grapevine samples during the vegetative growth stage. Samples 1–3 correspond to the white cultivar 'Ag Shani', and samples 4–6 correspond to the red cultivar 'Qara Shani'. Data are presented as mean ± SE of three independent biological replicates ($n = 3$). Each biological replicate represents an individual grapevine plant randomly selected from laboratory-confirmed GLRaV-3–infected vines, with a corresponding healthy control of the same cultivar and comparable age. All biochemical measurements were performed in technical triplicate for each biological replicate. Asterisks indicate statistically significant differences between infected and corresponding healthy control plants (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$), whereas ns denotes no significant difference

increase, $P < 0.001$), from 0.46 to 0.63 μmol mg^{-1} protein min^{-1} in sample 2 (≈37% increase;, $P < 0.01$), and from 0.25 to 0.48 μmol mg^{-1} protein min^{-1} in sample 3 (≈92% increase; $P < 0.001$). In 'Qara Shani', APX activity increased from 0.39 to 0.72 μmol mg^{-1} protein min^{-1} in sample 4 (≈85% increase; $P < 0.001$), from 0.28 to 0.54 μmol mg^{-1} protein min^{-1} in sample 5 (≈93% increase; $P < 0.001$), and from 0.32 to 0.58 μmol mg^{-1} protein min^{-1} in sample 6 (≈81% increase; $P < 0.001$). Notably, APX induction frequently approached a twofold increase, indicating strong activation of the ascorbate-dependent H_2O_2 detoxification pathway.

Comparatively, the relative increase in APX activity was generally greater than that observed for CAT, particularly in samples 3, 4, and 5, suggesting that the ascorbate–glutathione cycle plays a dominant role in ROS detoxification during GLRaV-3 infection. The more pronounced enzymatic activation in 'Qara Shani' further indicates cultivar-dependent differences in oxidative stress responsiveness.

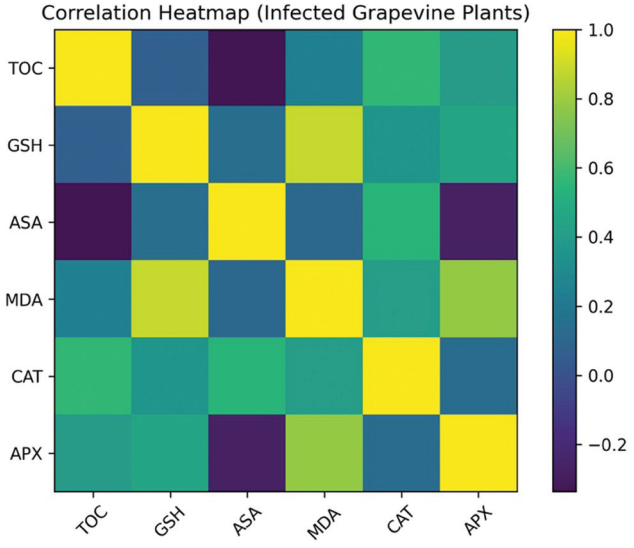

Fig. 5. Pearson correlation heatmap of oxidative stress and antioxidant parameters in grapevine leaves infected with Grapevine leafroll-associated virus 3. The matrix includes non-enzymatic antioxidants (ASA, GSH, TOC), lipid peroxidation marker (MDA), and antioxidant enzymes (CAT, APX). Correlation coefficients (Pearson's *r*) were calculated using biological replicates ($n = 3$). Color gradients indicate the strength and direction of correlations (positive to negative), with color intensity proportional to *r* values

To further elucidate the relationships among oxidative stress markers and antioxidant defense components, Pearson correlation analysis was conducted for all studied compounds. The resulting correlation matrix was visualized as a heatmap to illustrate the strength and direction of pairwise associations among all measured biochemical parameters **(Fig. 5)**.

The correlation heatmap revealed distinct relationships among enzymatic and non-enzymatic antioxidant components in GLRaV-3-infected grapevine plants. Ascorbic acid (ASA) exhibited a weak positive correlation with glutathione (GSH) ($r \approx 0.18$, $P < 0.05$), indicating that increases in ASA were only minimally associated with concurrent changes in GSH levels, suggesting limited coordination within the ascorbate–glutathione cycle under virus-induced oxidative stress. In contrast, ASA showed a moderate negative correlation with tocopherol (TOC) ($r \approx -0.34$, $P < 0.05$), highlighting a potential differential regulation of hydrophilic (ASA, GSH) versus lipophilic (TOC) antioxidant pools in response to viral infection. GSH was moderately positively correlated with APX activity ($r \approx 0.52$, $P < 0.05$), supporting a functional linkage between glutathione availability and enzymatic H_2O_2 detoxification, consistent with the central role of glutathione in sustaining APX-mediated redox cycling. Malondialdehyde (MDA) content demonstrated positive correlations with CAT ($r \approx 0.41$, $P < 0.05$) and APX activities ($r \approx 0.78$, $P < 0.05$), indicating that enhanced lipid peroxidation triggered a stress-responsive upregulation of enzymatic antioxidant defenses. Conversely, MDA showed weak or negative correlations with non-enzymatic antioxidants, specifically ASA ($r \approx 0.12$, $P > 0.05$), GSH ($r \approx -0.08$, $P > 0.05$), and TOC ($r \approx -0.15$, $P > 0.05$), reflecting that the soluble antioxidant system partially, but not fully, compensated for oxidative membrane damage.

DISCUSSION

The present study demonstrates that confirmed GLRaV-3 infection is associated with measurable alterations in both enzymatic and non-enzymatic components of the grapevine antioxidant system. In 2023, a total of 45 leaf samples were collected from three productive vineyards in the Salyan region, of which 13 tested positive for GLRaV-3, corresponding to a detection frequency of approximately 36.1%. While these results provide an indication of virus prevalence and its biochemical impact under local field conditions, the survey was limited in spatial and temporal

scope (single year, three vineyards). Therefore, the findings should be interpreted as preliminary evidence for the epidemiological and physiological relevance of GLRaV-3, and further multi-year, multi-site surveys are required to better characterize virus distribution and its broader impact on grapevine antioxidant responses.

At the biochemical level, infected plants exhibited a pronounced increase in lipid peroxidation, as reflected by elevated malondialdehyde (MDA) levels. The approximately 2.8–3.4-fold increase in MDA suggests that virus-induced reactive oxygen species (ROS) production exceeded basal detoxification capacity, resulting in measurable membrane damage (Bayramova et al., 2018; Bayramova et al., 2021; Bertamini et al., 2004). The accumulation of MDA confirms the establishment of oxidative stress during GLRaV-3 infection, as was also observed by Bertamini et al., 2005 and Vega et al., 2011. ROS generation is a well-recognized component of plant–pathogen interactions, functioning both as a signaling mechanism and as a potential cause of cellular injury when not adequately controlled (Xu et al., 2024; Szarka et al., 2012). The substantial increase in lipid peroxidation observed here indicates that oxidative pressure in infected leaves was sufficiently high to disrupt membrane integrity, despite concurrent activation of antioxidant systems (Ali et al., 2024; Carvalho et al., 2015; Caverzan et al., 2012).

In parallel with enhanced oxidative damage, infected plants displayed consistent increases in non-enzymatic antioxidants, including ASA, GSH, and TOC. In addition to its antioxidant function, ASA serves as a cofactor for various enzymes and acts as a regulatory molecule in phytohormone biosynthesis and redox signaling pathways (Guidi et al., 2021). Elevated ASA levels are frequently observed in stress-tolerant genotypes, supporting its role in plant growth, development, and adaptation to environmental and internal stress factors (Ali et al., 2024; Szarka et al., 2012). In plant cells, ASA is mainly synthesized in mitochondria and is distributed across multiple cellular compartments, with particularly high concentrations in chloroplasts (Kuźniak et al., 2017). It functions in concert with other antioxidants, particularly within the ascorbate–glutathione cycle, to maintain cellular redox homeostasis under stress conditions (Szarka et al., 2012; Edmund et al., 2024). As a key component of the antioxidant defense system, glutathione (GSH) directly detoxifies reactive oxygen species (ROS) and functions synergistically with ascorbate within the ascorbate–glutathione cycle. Alterations in GSH levels are widely recognized as indicators of plant responses to environmental and biotic stresses (Ali et al., 2024; Otulak-Kozieł et al., 2022; Edmund et al., 2024). The observed increase in GSH across infected samples indicates reinforcement of thiol-dependent redox buffering, whereas the moderate but consistent elevation of ASA suggests enhanced capacity for peroxidase-mediated H_2O_2 reduction. The accumulation of total tocopherols further indicates strengthening of membrane-associated antioxidant protection (Sahu et al., 2022). Tocopherols are lipophilic antioxidants that protect polyunsaturated fatty acids from peroxidation and stabilize membrane structure under stress conditions (Sgherri et al., 2013). Although individual tocopherol isoforms (α-, β-, γ-, and δ-tocopherols) and tocotrienols were not quantified separately, the increase in total TOC content suggests an adaptive adjustment aimed at limiting oxidative damage within lipid compartments. Tocopherols are known to participate in plant responses to salinity, drought, heavy metal toxicity, ozone exposure, and UV radiation, and are also associated with physiological processes affecting plant growth and productivity (Faizan et al., 2023; Szarka et al., 2012).

Nevertheless, the persistence of elevated MDA levels as determined by us implies that lipophilic antioxidant reinforcement was not sufficient to fully counteract ROS-induced membrane injury as was determined for fava bean (*Vicia faba*) under bean yellow mosaic virus infection (Hamzah et al., 2021).

Enzymatic antioxidant responses were also markedly stimulated. Both catalase (CAT) and ascorbate peroxidase (APX) activities increased significantly in infected plants. CAT decomposes hydrogen peroxide directly into water and oxygen, whereas APX reduces H_2O_2 using ascorbate as an electron donor. The simultaneous upregulation of APX activity together with increased ASA and GSH levels is consistent with activation of the ascorbate-dependent H_2O_2 detoxification pathway (Caverzan et al., 2012; Chew et al., 2003; Kuźniak et al., 2017). In several infected samples, APX activity approached nearly twofold increases relative to controls, indicating strong stimulation of enzymatic ROS-scavenging capacity. CAT activity also showed substantial enhancement, further supporting intensified hydrogen peroxide turnover. Similar increases in antioxidant enzyme activities have been documented in other plant–virus systems, including

infections caused by *Cotton leaf curl burewala virus*, *Telosma mosaic virus*, *Tomato mosaic virus* and *Pepper mild mottle virus* (Siddique et al., 2014; Sahu et al., 2022; Perez-Bueno et al., 2006; Shopova et al., 2020; Rehman et al., 2021).

Despite this coordinated antioxidant activation, oxidative damage remained elevated, as evidenced by sustained high MDA concentrations. This finding suggests that antioxidant upregulation represents a compensatory response rather than a fully protective mechanism. In other words, GLRaV-3 infection appears to induce a state of redox imbalance characterized by simultaneous ROS accumulation and activation of detoxification pathways, with the latter partially — but not completely — offsetting oxidative injury.

Differences observed between the white cultivar ('Ag Shani') and the red cultivar ('Qara Shani') indicate potential genotype-dependent variation in antioxidant responsiveness. In several parameters, the red cultivar exhibited stronger induction of both enzymatic and non-enzymatic antioxidants, accompanied by more pronounced increases in MDA. However, due to the limited number of biological replicates ($n = 3$) and field-based sampling design these differences should be interpreted as indicative rather than conclusive evidence of genotype-dependent redox regulation. Since this study focused on determination of total amounts and their differences of biochemical indicators of oxidative stress and antioxidant status rather than on mechanistic or transcriptional regulation. our findings should be interpreted as documenting infection-associated redox adjustments rather than defining specific regulatory pathways. Future studies incorporating larger sample sizes, controlled experimental conditions, quantitative viral load assessment, and time-course analyses will be necessary to clarify the dynamics of redox regulation and the extent of genotype-dependent variability during GLRaV-3 infection.

Previous studies have reported similar and other alterations in antioxidant enzyme activities, including APX, during plant–virus interactions in the grapevine-GLRaV-3 disease complex (Bayramova et al., 2018 and 2021 Bayramova et al., 2023; Huseynova et al., 2016; Sultanova et al., 2019; Sultanova et al., 2025). In the present study, GLRaV-3 infection was again associated with increased APX activity together with elevated levels of ASA and GSH. These changes indicate adjustments in the antioxidant system under viral stress conditions. ASA and GSH function cooperatively in cellular redox buffering, and concurrent increases in both metabolites, together with enhanced APX activity, are consistent with activation of the ascorbate-dependent H_2O_2 detoxification pathway (Chew et al., 2003; Caverzan et al., 2012; Moutinho-Pereira et al., 2012; Kuźniak et al., 2017).

However, the present study did not investigate the regulation of this pathway at the molecular level, and therefore conclusions are limited to observed biochemical changes. Our findings demonstrate that GLRaV-3 infection is associated with coordinated modifications in both enzymatic (APX, CAT) and non-enzymatic (ASA, GSH, TOC) components of the antioxidant system in grapevine leaves. In the present study, GLRaV-3 infection was associated with concurrent increases in TOC, ASA, and GSH levels, together with elevated activities of antioxidant enzymes.

Overall, the substantial and statistically significant elevation of both CAT and APX activities demonstrates a robust enzymatic antioxidant response to GLRaV-3 infection. However, when considered together with the elevated MDA levels observed in parallel, these findings suggest that enzymatic upregulation represents a compensatory mechanism that mitigates—but does not fully prevent—virus-induced oxidative damage. The observed correlation patterns suggest that, in the sampled GLRaV-3–infected grapevine leaves, glutathione availability was moderately linked with enzymatic H_2O_2 detoxification (APX activity), while associations between non-enzymatic antioxidants and lipid peroxidation were weak or variable. These results indicate that, under viral stress, enzymatic and non-enzymatic antioxidant components may respond differentially, with enzymatic defenses showing a more consistent activation in response to oxidative damage than the soluble antioxidant pool. However, given the limited number of biological replicates analyzed, these findings should be interpreted as indicative of potential redox adjustments rather than conclusive evidence of systemic network regulation.

CONCLUSIONS

The present study evaluated changes in lipid peroxidation and antioxidant defense parameters in grapevine leaves of two cultivars, 'Ag Shani' (white) and 'Qara Shani' (red), with confirmed GLRaV-3 infection. Infected plants exhibited significantly higher

malondialdehyde (MDA) levels, indicating increased lipid peroxidation. Specifically, MDA increased by approximately 95–121% in the white cultivar and 50–121% in the red cultivar compared with healthy controls ($P < 0.01$–0.001). Enzymatic antioxidant activities were markedly elevated: catalase (CAT) activity increased by 30–66% in 'Ag Shani' and 54–69% in 'Qara Shani', while ascorbate peroxidase (APX) activity increased by 37–92% in the white cultivar and 81–93% in the red cultivar. These changes indicate strong activation of the H_2O_2-scavenging pathway under viral stress. Non-enzymatic antioxidants were also enhanced in infected leaves. Glutathione (GSH) increased by 24–33% in 'Ag Shani' and 50–56% in 'Qara Shani', ascorbic acid (ASA) increased by 16–35% in the white cultivar and 10–17% in the red cultivar, and total tocopherol (TOC) increased by 12–25% in 'Ag Shani' and 19–50% in 'Qara Shani'. Collectively, these findings indicate that GLRaV-3 infection induces measurable oxidative stress in grapevine leaves, characterized by enhanced lipid peroxidation and a coordinated upregulation of both enzymatic and non-enzymatic antioxidant defense components. The magnitude of these responses, particularly in the red cultivar, highlights cultivar-specific differences in oxidative stress management during viral infection.

Although similar responses have been described in other plant–virus systems, the present study provides biochemical data for GLRaV-3–infected grapevine cultivars under local field conditions. Given the limited number of biological replicates, the results should be interpreted as indicative of infection-associated redox adjustments rather than evidence of specific regulatory mechanisms. Further studies involving larger sample sizes and controlled experimental designs will be required to clarify genotype-dependent variability and to better understand the functional significance of antioxidant responses during GLRaV-3 infection.

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УДК 634.8:615.9

ЕНДОГЕННІ АНТИОКСИДАНТНІ ЗАХИСНІ ВІДПОВІДІ ВИНОГРАДНИХ РОСЛИН НА ІНФЕКЦІЮ GLRAV-3

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Мета. Метою даного дослідження було вивчити, як вірусні інфекції, що суттєво погіршують ріст і врожайність рослин, впливають на накопичення активних форм кисню (АФК), а також оцінити роль як ферментативних антиоксидантних захисних механізмів, так і неферментативних низькомолекулярних антиоксидантів, зокрема аскорбату (ASA), глутатіону (-L-глутаміл-L-цистеїніл-гліцину = GSH) та токоферолів (TOC), у пом'якшенні вірусоіндукованого окислювального стресу в рослинах. **Методи.** У цьому дослідженні було зібрано загалом 45 зразків листя зі зрілих 10-річних лоз сортів "Гара Шані" (червоний) та "Аг Шані" (білий). Відбір зразків проводили у трьох виноградниках, де з кожного виноградника відбирали п'ять лоз, а з кожної лози — три фізіологічно зрілих листки, що дало 15 листків із кожного виноградника та 45 листків загалом (3 виноградники × 5 лоз × 3 листки). На кожному винограднику одну лозу без видимих симптомів було визначено як здоровий контроль, тоді як решта лоз виявляли типові вірусні симптоми, такі як почервоніння країв листя та черешків, зниження життєздатності, скручування листя, пожовтіння та скручування. Відповідно, було отримано 36 листків із лоз із симптомами та 9 листків із лоз без симптомів (здорових). Інфікування вірусом скручування листя винограду 3 (GLRaV-3) було підтверджено у зразках винограду за допомогою експрес-тесту AgriStrip, подвійного антитілового сендвіч-ІФА (DAS-ELISA) та зворотної транскрипції-полімеразної ланцюгової реакції (RT-PCR). Згодом вірусоіндуковані зміни в неферментативних антиоксидантах були кількісно оцінені за допомогою спектрофотометричного визначення аскорбату та глутатіону, токоферолів за допомогою реакції Еммері–Енгеля, а також перекисного окислення ліпідів через вміст МДА за допомогою аналізу речовин, що реагують з тіобарбітуровою кислотою (TBARS). Ферментативну антиоксидантну активність, включаючи

аскорбатпероксидазу та каталазу, визначали спектрофотометрично, а вміст розчинного білка встановлювали за методом Седмака. **Результати.** Вірусний скринінг за допомогою тестів AgriStrip та DAS-ELISA, а потім RT-PCR, спрямованої на ген білка оболонки, підтвердив інфікування GLRaV-3 у 13 зразках (приблизно 36,1%), тоді як безсимптомні лози дали негативний результат, що продемонструвало узгодженість між візуальною оцінкою та молекулярною діагностикою. Інфікування GLRaV-3 призвело до значного підвищення рівнів неферментативних антиоксидантів порівняно зі здоровими контролями. Вміст токоферолу (TOC) збільшився на 18–73% у заражених зразках 2–6, тоді як зразок 1 не показав значних змін. Рівні глутатіону (GSH) підвищилися на 20–60% у заражених зразках 1, 2, 4, 5 та 6, тоді як зразок 3 не показав значної різниці. Вміст аскорбінової кислоти (ASA) збільшився на 10–23% у заражених зразках 1, 3, 5 та 6, тоді як у зразках 2 та 4 не було виявлено значущої різниці. Пероксидація ліпідів, про що свідчить вміст малонового діальдегіду (MDA), також була значно підвищеною в заражених листках. Рівні МДА збільшилися приблизно в 1,5–2,6 раза у зразках 1, 2, 4, 5 та 6 порівняно зі здоровими контролями, досягаючи абсолютних значень 0,85–1,75 мкмоль/г свіжої маси проти 0,45–0,9 мкмоль/г свіжої маси у контролях. Паралельно з цим підвищилася активність ключових антиоксидантних ферментів: активність каталази (CAT) зросла на 25–48%, а активність аскорбатпероксидази (APX) — на 30–55% в інфікованих листках порівняно з відповідними контролями. **Висновки.** Дослідження, проведені у виноградниках Саляну, підтвердили інфікування GLRaV-3, що підтверджує зв’язок між спостережуваними симптомами та біохімічними реакціями на окислювальний стрес. Ці біохімічні зміни свідчать про те, що інфікування GLRaV-3 викликає окислювальний стрес і одночасно активує як ферментативні, так і неферментативні антиоксидантні захисні механізми у листі винограду. Скоординоване підвищення рівнів ASA, GSH, TOC, CAT та APX, поряд із підвищеним рівнем MDA, вказує на складну редокс-регуляцію, спрямовану на пом’якшення окислювального пошкодження, спричиненого вірусом.

Ключові слова: виноград; віруси; метаболіти; антиоксидантні ферменти.