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RESEARCH PAPER

Antioxidant enzyme activities in chili plants in response to the infection of *Pepper yellow leaf curl Indonesia virus*

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ABSTRACT

Chili pepper is an important agricultural crop but is highly vulnerable to viral diseases, including *Pepper yellow leaf curl Indonesia virus* (PepYLCIV). This study investigated the biochemical response of chili plants to PepYLCIV infection by examining changes in antioxidant enzyme activities: peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). The effects of sample handling methods on enzyme activity measurements were also evaluated. The experiment was conducted in a biosafety greenhouse using a factorial design with four replications. The treatments included two chili varieties (*Bara* and *Bonita*), plant condition (healthy and PepYLCIV-infected), sample types (fresh leaf tissue and frozen leaf tissue stored at −80 °C), and seven sampling times (1, 3, 5, 7, 14, 21, and 28 days post-inoculation). Result showed that POD activity was significantly higher in infected plants than in healthy plants, whereas CAT and APX activities showed no significant differences between plant health conditions. Fresh samples consistently exhibited higher enzyme activity than frozen samples. POD and CAT activities peaked at 28 days post-inoculation, while APX activity was the highest at 5 days post-inoculation and fluctuated over time. These findings highlight the importance of considering infection status, sampling time, and sample processing when evaluating antioxidant enzymes in plant–virus interaction studies.

Keywords: antioxidant enzymes, ascorbate peroxidase, *Capsicum annuum*, catalase, PepYLCIV

INTRODUCTION

Indonesia is one of the leading chili pepper–producing countries in the world, however, productivity is often constrained by various plant diseases. Among these, pepper yellow leaf curl disease (PYLCD) is one of the most destructive. The disease is caused by *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), which was first reported in Indonesia in 1999 and has since spread widely across major production areas, including Java, Bali, and Sumatra (Hidayat et al., 1999; Sulandari, 2004; Jamsari & Pedri, 2013; Neriya et al., 2020). PepYLCIV is primarily transmitted by the whitefly (*Bemisia tabaci*) and may also spread through grafting (Inoue-Nagata et al., 2007; Koeda et al., 2018). Typical symptoms include yellowing, curling, and deformation of leaves (Paradisa et al., 2022; Wahyono et al., 2023). In severe cases, the disease can result in near-complete yield loss, highlighting the urgent need for effective management strategies.

Viral infection disrupts plant physiological processes, including growth and metabolism, often triggering oxidative stress and increasing reactive oxygen species (ROS) production (Hasanuzzaman et al., 2020). Although excessive ROS can damage cellular structures, ROS accumulation also activates plant defense signaling pathways, including pathogenesis-related genes and antioxidant enzymes

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(Negi et al., 2016; Xu et al., 2024). Key enzymes such as catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) protect cells by detoxifying ROS and maintaining cellular homeostasis during infection (Jaiswal et al., 2013; Xu et al., 2024). Additionally, polyphenol oxidase (PPO) and POD contribute to the biosynthesis of phenolic compounds, which possess antioxidant and antimicrobial properties. Increased POD activity has been associated with phenolic deposition in cell walls during incompatible plant–microbe interactions (El-Argawy & Adss, 2016), and may further initiate systemic responses including localized necrosis (Simons & Ross, 1971).

Similar patterns of antioxidant response have been observed in studies involving other plant–pathogen systems. Resistant potato cultivars infected with *Ralstonia solanacearum* exhibited higher expression of POD and PPO compared to susceptible cultivars (El-Argawy & Adss, 2016). Likewise, APX expression increased significantly in sugarcane cultivar PS881 infected by multiple viruses (Neliana et al., 2024). In chili plants infected with leaf curl viruses, activities of POD, PPO, APX, and SOD were consistently higher in resistant genotypes compared to susceptible ones (Kingkampang et al., 2020; Sran et al., 2023). CAT activity has also been shown to increase in whitefly-infested tobacco plants, contributing to enhanced resistance against aphids (*Myzus persicae*) (Zhao et al., 2016). Collectively, these findings suggest that antioxidant enzymes serve as potential biochemical markers associated with virus resistance in plants (Sran et al., 2023).

Responses of antioxidant enzymes to PepYLCIV infection may vary among chili pepper genotypes and environmental conditions. Therefore, this study aimed to evaluate antioxidant enzyme activity in two Indonesian chili pepper varieties, *Bara* and *Bonita*, following PepYLCIV infection. Enzyme activity was monitored at multiple time points post-infection to assess temporal variation in response. The results are expected to enhance understanding of host defense mechanisms and contribute to breeding and management strategies aimed at improving resistance to PepYLCIV.

MATERIALS AND METHODS

Research Site. This research was conducted at the Biosafety Greenhouse Facility and the Genomic Laboratory, National Research and Innovation Agency (BRIN), Cibinong, West Java, from June to September 2024. A factorial experimental design with four replications was used to evaluate the effects of different treatments on antioxidant enzyme activity. The first factor was plant variety, consisting of *Bara* and *Bonita*, selected to compare varietal response differences. The second factor was plant condition (healthy and PepYLCIV-infected plants) to assess the impact of infection. The third factor was sample type, with both fresh and frozen leaf samples analyzed to determine the effect of sample storage conditions. Sampling was conducted at 1, 3, 5, 7, 14, 21, and 28 days post-infection (dpi). Enzyme activity was measured using a Varioskan™ LUX Multimode Microplate Reader.

PepYLCIV Transmission in Chili Plants. Seeds of *Bara* (*Capsicum annuum*) and *Bonita* (*Capsicum frutescens*) were germinated in seed trays containing moist tissue papers. After 10 days, seedlings were transferred to trays containing a soil–goat manure mixture (2 : 1). At three weeks after germination, seedlings were transplanted into 20 × 20 cm polybags filled with the same growing medium. Virus transmission was carried out using *B. tabaci* (whiteflies) on four-week-old plants following Ganefianti et al. (2017). Ten viruliferous whiteflies per plant were allowed a 24-hour acquisition feeding period on source-infected plants and subsequently transferred to healthy plants for a 24-hour inoculation feeding period.

Disease severity (DS) was assessed using the scoring categories described by Ganefianti (2010): 0 = no symptoms; 1 = mild mosaic; 2 = mosaic, yellow streaks, leaf curling; 3 = mosaic, yellow streaks, curling, growth distortion; 4 = severe symptoms, contrasting streaks, severe deformation, and stunted growth. DS was calculated using the formula:

$$DS = \frac{\sum_{i-1}^{k} v_i \times n_i}{N \times V} \times 100\%$$

DS = Disease severity (%);
ni = Number of plants in category i;
vi = Numerical score of category;
N = Total number of assessed plants;
V = Highest category score.

Disease incidence was calculated using:

$$DI = \frac{n}{N} \times 100\%$$

DI = Disease incidence (%);
n = Number of symptomatic plants;
N = Total number of observed plants.

Plant resistance classification followed Ganefianti (2010): highly resistant (0–1%), resistant (1–5%), moderately resistant (5–10%), moderately

susceptible (10–20%), susceptible (20–40%), and highly susceptible (>40%).

Sample Collection. Leaves were collected at 1, 3, 5, 7, 14, 21, and 28 dpi for enzyme analysis. Fresh samples were analyzed immediately, while frozen samples were stored at -80 ºC and analyzed within seven days.

Molecular Detection. DNA was extracted using a modified CTAB method (Paradisa et al., 2024). PCR reactions were performed using MyTaq HS Red Mix, 2× (Bioline, United Kingdom). Each 10 µL reaction consisted 5 µL MyTaq HS Red Mix, 1 µL DNA (100 ng/µL), 0.5 µL of each primer, and 3 µL of nuclease-free water.

DNA-A detection used primers SPG1-F (5'-CCCCKGTGCGWRAATCCAT-3') and SPG2-R (5'-ATCCVAAYWTYCAGGGAGCTAA-3') (Li et al., 2004). Cycling conditions: initial denaturation at 94 ºC for 3 min, 35 cycles of 94 ºC for 1 min, 59 ºC for 1 min, and 72 ºC for 2 min; final extension at 72 ºC for 10 min.

DNA B detection used primers from Koeda et al. (2018): DNA-B-F (5'-TGTCCTCATCGTAGTCACA-CA-3')and DNA-B-R (5'-GAAGATAGTCTGTAC-CGTCATGTAC-3'). Cycling conditions: 94 ºC for 2 min, 45 cycles of 94 ºC for 30 sec, 68 ºC for 30 sec, and 72 ºC for 1 min, final extension at 72 ºC for 3 min.

PCR products were separated on 1% agarose gel stained with 0.004% FluoroSafe DNA Stain (1st BASE, Singapore).

Enzyme Extraction. Antioxidant enzyme extraction followed Salsinha et al. (2023) with modifications. Approximately 100 mg leaf tissue was homogenized in 1 mL of 50 mM potassium phosphate buffer (pH 7) containing 1 mM EDTA and 1% PVP. Samples were centrifuged at 12,000 g for 20 min at 4 ºC, and extracts were kept on ice during biochemical assays.

Enzyme Activity Assays.

Peroxidase (POD). POD activity measured following Zahir et al. (2021) with adaptation for microplate format. Each well contained 10 µL enzyme extract, 10 µL guaiacol (1.5%), and 250 µL phosphate buffer. The reaction was initiated with 10 µL of 300 mM H_2O_2, and absorbance was read at 470 nm. One unit of POD activity was defined as the amount of enzyme required to oxidize 1 nmol of guaiacol per minute.

Catalase (CAT). CAT activity was determined using Zahir et al. (2021). Each well contained 10 µL enzyme extract, 250 µL of 50 mM phosphate buffer pH 7, and 10 µL of 300 mM H_2O_2. Absorbance was read at 240 nm. One CAT unit represented the decomposition of 1 nmol H_2O_2 per minute.

Ascorbate Peroxidase (APX). APX activity followed the method of Nakano & Asada (1981) with modifications. Each well contained 10 µL enzyme extract, 250 µL of 50 mM phosphate buffer pH 7, and 10 µL ascorbic acid (7.5 mM), and 10 µL of 300 mM H_2O_2. Absorbance was measured at 290 nm at 30 ºC.

Enzyme activity was calculated using formula:

$$\text{Enzyme activity (in mM/g fresh weight)} = \frac{(\text{activity} \times A \times \left(\frac{V}{a}\right)}{E \times W}$$

A = Assay reaction volume;
V = Total extraction buffer volume;
a = Enzyme extract volume used;
W = Fresh weight of sample;
E = extinction coefficient: POD (25.5 mM/cm), CAT (39.4 mM/cm), APX (2.8 mM/cm).

The optical density was measured at 470 nm for POD and at 240 nm for CAT. Meanwhile, APX was measured at 290 nm at 30 ºC.

Data Analysis. Enzyme activity data were analyzed using ANOVA. Where significant differences were detected, post-hoc comparisons were conducted using Duncan's Multiple Range Test at a 5% significance level. Statistical analyses were performed using IBM SPSS Statistics version 23.

RESULTS AND DISCUSSION

Symptom Observation of PepYLCIV Infection. Plant resistance to abiotic and biotic stress largely depends on their capacity to regulate reactive oxygen species (ROS). ROS, including superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radicals, are natural by-products of essential metabolic pathways, such as respiration and photosynthesis (Bhattacharjee, 2019). Under stress, ROS accumulation increases and may lead to oxidative damage and programmed cell death. Antioxidant enzymes play key roles in mitigating ROS damage and supporting plant survival under stress conditions (Hasanuzzaman et al., 2020).

PepYLCIV infection has been widely reported in chili-growing regions in Indonesia (Paradisa et al., 2022; Santosa et al., 2024). Symptoms of PepYLCIV infection include yellowing and curling of leaves, particularly the upward curling of young leaves, as observed in both *Bara* and *Bonita* (Figure 1B, 1D).

The leaf veins may also undergo chlorosis, becoming transparent or yellow. In addition, the leaves—especially young ones—were curled upward, shrunken, stunted, and exhibited a green and yellow mosaic pattern. The infection affected overall plant growth, as indicated by growth retardation and failure of flowers to set fruit. Ultimately, infected plants became yellow and severely stunted.

Molecular Detection of PepYLCIV. In addition to symptom observation, identification of PepYLCIV was also performed molecularly. Amplification using the SPG primer produced a DNA band of approximately 912 bp (Figure 2A). This result confirms PepYLCIV infection in both *Bara* and *Bonita*, as the SPG primer targets the open reading frame (ORF) AC2 and ORF AC1 regions of Begomovirus DNA-A (Li et al., 2004). Further amplification using the PepYLCIV DNA-B primer produced a 385 bp fragment (Figure 2B), corresponding to the common region and part of the BV1 ORF of the PepYLCIV genome.

Host Response Evaluation Based on Disease Severity and Incidence. The chili pepper varieties *Bara* and *Bonita* were used in this study. *Bara* is known to be highly susceptible to PepYLCIV and is commonly used as a susceptible control in resistance screening trials. Meanwhile, *Bonita* also showed a susceptible response in previous screening tests (data not shown). This is supported by the results of virus inoculation using whiteflies, which produced disease severity and incidence values of 29.38% and 60% in *Bara*, and 38.13% and 62.5% in *Bonita*, respectively (Table 1). Based on these measurements, both varieties are categorized as susceptible, although disease severity and incidence were slightly higher in *Bonita*. These findings differ slightly from those of Sandra et al. (2022), who reported that *Bonita* was moderately susceptible, and from Sayekti et al. (2021), who classified it as moderately resistant to PepYLCIV. Meanwhile, *Bara* consistently remains categorized as highly susceptible (Ayu et al., 2021).

The activity of peroxidase (POD), catalase

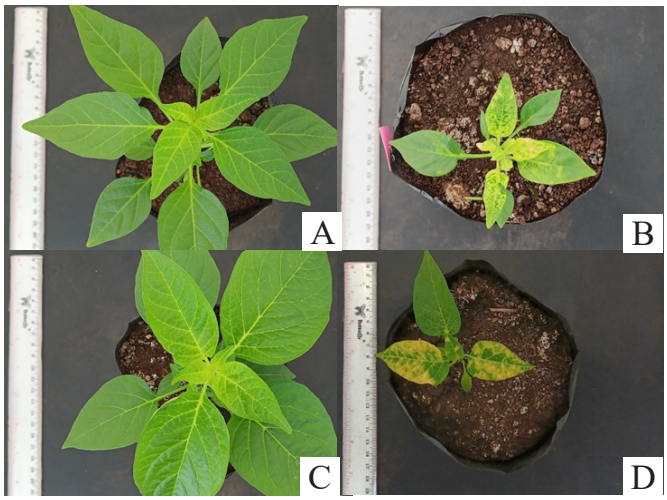

Figure 1. Symptoms of PepYLCIV infection in chili plants at 4 weeks post inoculation. A. *Bara* variety without PepYLCIV infection (healthy); B. *Bara* variety infected with PepYLCIV; C. *Bonita* variety without PepYLCIV infection (healthy); D. *Bonita* variety infected with PepYLCIV.

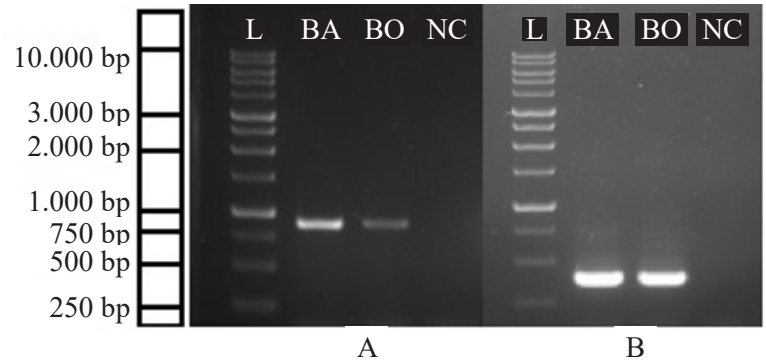

Figure 2. Amplification of DNA-A (A) and DNA-B (B) of PepYLCV from *Bara* (BA) and *Bonita* (BO) at 4 weeks post-inoculation. L = 1 kb DNA ladder; NC = Negative Control.

(CAT), and ascorbate peroxidase (APX) are important indicators for assessing plant responses to stress conditions. In this study, enzyme activities were measured in healthy and infected plants, and in both fresh and frozen samples, at several sampling times. Analysis of variance (ANOVA) results (Table 2) showed that the examined factors significantly influenced POD, CAT, and APX activities. Plant condition (factor B) had a significant effect on POD activity, while sample type (factor C) significantly affected CAT and APX activities. Sampling time (factor D) significantly influenced all three enzymes. The interaction between plant condition and sampling time (B × D) significantly affected POD activity, while the interaction between sample type and sampling time (C × D) significantly affected CAT activity. These findings indicate that both individual factors and interactions between factors influence enzyme activity, likely reflecting the plant's biochemical responses to infection and environmental conditions.

Further analysis using Duncan's test (α = 0.5%) is presented in Table 3. The results showed that POD activity was higher in infected plants than in healthy plants. However, no significant differences were observed between healthy and infected plants in CAT and APX activity. Sample type also influenced enzyme activity, with fresh samples exhibiting higher CAT and APX activity than frozen samples. POD activity did not show significant differences between fresh and frozen samples. Sampling time also had an effect: POD activity increased significantly at 28 dpi and continued to show a gradual increase each week until that point. CAT activity peaked at 28 dpi but fluctuated from week to week. APX activity showed a significant difference at 5 dpi and, similar to CAT, fluctuated across sampling times.

Table 4 presents the activity of POD, CAT, and APX in healthy and infected plants across various sampling times. In healthy plants, POD activity ranged from 0.0805 to 0.1575, with the highest value at 5 dpi and the lowest at 21 dpi. CAT activity ranged from 0.1131 to 0.2027, with the highest activity at 28 dpi and the lowest at 3 dpi. APX activity ranged from 1.1357 to 1.9089, with the highest value at 28 dpi and the lowest at 14 dpi.

In infected plants, POD activity ranged from

Table 1. Disease severity (DS) and disease incidence (DI) in chili plants at 28 days post-inoculation

Variety	DS (%)	DI (%)	Plant resistance category
Bara	29.375	60	Susceptible
Bonita	38.125	62.5	Susceptible

Table 2. Result of analysis of variance (ANOVA) of enzyme activity

Source	Degree of freedom	Mean square		
		POD	CAT	APX
Variety (A)	1	0.022	0.001	0.0000010
Plant condition (B)	1	3.791 *	0.006	0.045
Sample type (C)	1	0.014	0.014 *	7.577 *
Sampling time (D)	6	1.033 *	0.017 *	3.843 *
A × B	1	0.156	0.004	0.316
A × C	1	0.086	0.000	0.000
A × D	6	0.062	0.005	0.509
B × C	1	0.014	0.005	0.230
B × D	6	1.133 *	0.004	1.143
C × D	6	0.034	0.013 *	2.667
A × B × C	1	0.048	0.0000939	0.000
A × B × D	6	0.108	0.002	0.255
A × C × D	6	0.017	0.004	0.228
B × C × D	6	0.032	0.001	0.228
A × B × C × D	6	0.014	0.002	0.141
Error	168	0.175	0.003	1.612

* = significant at α 0.5%.

0.1428 to 0.5250, with the highest value at 21 dpi and the lowest at 7 dpi. CAT activity ranged from 0.1194 to 0.1681, with the highest activity at 14 dpi and the lowest at 21 dpi. APX activity ranged from 1.0606 to 2.5178, with the highest value at 5 dpi and the lowest at 21 dpi. Overall, enzyme activity varied significantly depending on plant health condition and sampling time. POD and APX activities were generally higher in diseased plants compared to healthy plants, particularly at 21 dpi for POD and 5 dpi for APX. CAT activity

Table 3. Effects of plant condition, sample type, and sampling time on antioxidant enzymes activities

Plant condition	POD	CAT	APX
Healthy	0.1243 a	0.1566	1.6204
Infected	0.3844 b	0.1467	1.6488
Sample type			
Fresh	0.2622	0.1595 a	1.8186 a
Frozen	0.2466	0.1437 b	1.4506 b
Sampling time			
1 dpi	0.1380 a	0.1192 a	1.6061ab
3 dpi	0.1603 a	0.1256 ab	1.8154 ab
5 dpi	0.1555 a	0.1669 cd	2.1539 b
7 dpi	0.1375 a	0.1493 bc	1.7688 ab
14 dpi	0.2511 a	0.1749 cd	1.1374 a
21 dpi	0.3028 a	0.1472 bc	1.2516 a
28 dpi	0.6357 b	0.1783 d	1.7092 ab

POD = Peroxidase; CAT = Catalase; APX = Ascorbate Peroxidase. dpi = days post-infection. Numbers followed by the same letter in the same column for each treatment showed no significant difference based on the Duncan's test at α=5%.

Table 4. Effect of the interaction between plant condition and sampling time on antioxidant enzymes activity

Plant Condition	Sampling time	Activity (mM/gFW)		
		POD	CAT	APX
Healthy	1 dpi	0.1121 a	0.1188	1.5681
	3 dpi	0.1349 a	0.1131	1.8022
	5 dpi	0.1575 a	0.1686	1.7898
	7 dpi	0.1323 a	0.1500	1.6958
	14 dpi	0.1332 a	0.1817	1.1357
	21 dpi	0.0805 a	0.1613	1.4425
	28 dpi	0.1197 a	0.2027	1.9089
Infected	1 dpi	0.1638 a	0.1194	1.6440
	3 dpi	0.1856 a	0.1383	1.8287
	5 dpi	0.1533 a	0.1653	2.5178
	7 dpi	0.1428 a	0.1486	1.8419
	14 dpi	0.3688 a	0.1681	1.1391
	21 dpi	0.5250 a	0.1329	1.0606
	28 dpi	1.1516 b	0.1540	1.5093

POD = Peroxidase; CAT = Catalase; APX = Ascorbate Peroxidase; dpi = days post-infection. Numbers followed by the same letter in the same column for each treatment showed no significant difference based on the Duncan's test at α=5%.

showed more irregular patterns but still demonstrated notable time-dependent fluctuations.

Table 5 shows the influence of the interaction between sample type and sampling time. In fresh samples, POD activity ranged from 0.13 to 0.70, with the highest value at 28 dpi and the lowest at 5 dpi. In frozen samples, POD activity ranged from 0.20 to 0.60, with the highest value at 28 dpi and the lowest at 7 dpi. These results suggest that although enzyme activity decreases during storage, leaves preserved for up to one week still have potential for valid POD analysis.

CAT activity in fresh samples varied from 0.0950 to 0.1930, with the highest value at 28 dpi and the lowest at 3 dpi. In contrast, CAT activity in samples stored at −80 °C ranged from 0.1158 to 0.1673, with the highest activity at 14 dpi and the lowest at 21 dpi. This indicates that CAT activity fluctuates depending on plant condition and storage treatment.

APX activity in fresh samples ranged from 1.4052 to 2.4601, with the highest value at 5 dpi and the lowest at 14 dpi. The elevated APX activity at 5 dpi may be associated with the early oxidative stress response, during which APX plays a key role in detoxifying H_2O_2 generated during metabolic processes. In frozen samples, APX activity ranged from 0.8696 to 2.0052, with the highest value at 28 dpi and the lowest at 14 dpi. These results indicate that sampling time significantly affects enzyme activity and that fresh samples consistently exhibit higher enzyme activity than frozen samples, likely due to enzymatic degradation during storage at −80 ºC.

Overall, enzyme activity showed significant variation depending on sample type and sampling time. POD and APX activities were generally higher in fresh samples than in frozen samples. CAT activity peaked in fresh samples at 28 dpi and in frozen samples at 14 dpi. Sampling time also contributed to variation in enzyme activity, with specific peak points observed for each enzyme and sample condition.

Peroxidase is a glycoprotein synthesized in the endoplasmic reticulum and then transported through the Golgi apparatus to the extracellular space or vacuoles, where it uses substrates such as hydrogen peroxide to carry out redox reactions (Jovanović et al., 2018). This enzyme plays roles in lignification, cell elongation, defense against stress, and seed germination (Shigeto & Tsutsumi, 2016). In enzyme activity tests, peroxidase causes a color change from orange to reddish-brown by oxidizing the substrate guaiacol into tetraguaiacol (de Oliveira et al., 2021). In both infected *Bara* and *Bonita* plants, POD activity was higher than in healthy plants. This finding is consistent with the results of Sran et al. (2023), where chili plants infected with yellow leaf curl disease showed increased POD activity compared to healthy plants. Higher enzymatic activity in diseased plants indicates activation of

Table 5. Effect of the interaction between sample type and sampling time on antioxidant enzymes activity

Sample type	Sampling time	Activity (mM/gFW)		
		POD	CAT	APX
Fresh	1 dpi	0.1256	0.1144 cd	1.6079
	3 dpi	0.1574	0.0950 d	1.9066
	5 dpi	0.1215	0.1825 ab	2.4601
	7 dpi	0.1341	0.1709 abc	2.3935
	14 dpi	0.2985	0.1826 ab	1.4052
	21 dpi	0.3051	0.1784 ab	1.5438
	28 dpi	0.6929	0.1930 a	1.4130
Frozen	1 dpi	0.1503	0.1238 bcd	1.6042
	3 dpi	0.1631	0.1564 abcd	1.7243
	5 dpi	0.1892	0.1514 abcd	1.8475
	7 dpi	0.1410	0.1277 bcd	1.1442
	14 dpi	0.2035	0.1673 bcd	0.8696
	21 dpi	0.3004	0.1158 cd	0.9593
	28 dpi	0.5784	0.1636 abc	2.0052

POD = Peroxidase; CAT = Catalase; APX = Ascorbate Peroxidase. dpi = days post-infection. Numbers followed by the same letter in the same column for each treatment showed no significant difference based on the Duncan's test at α=5 %.

defense mechanisms against PepYLCIV infection. The breakdown of H_2O_2 into water helps reduce oxidative stress and prevents cellular injury caused by H_2O_2 accumulation (Jovanović et al., 2018).

Catalase is a tetrameric enzyme composed of four identical polypeptide chains, each containing a heme group, with a molecular weight ranging from 54 to 60 kDa (Baker et al., 2023). The heme group contains an iron atom essential for the enzyme's ability to break down hydrogen peroxide into water and oxygen, thereby reducing oxidative stress in cells (Rajput et al., 2021; Sahoo & Tiwari, 2022). CAT activity is commonly measured using spectrophotometry by monitoring the decrease in absorbance of hydrogen peroxide (H_2O_2) at approximately 240 nm. As CAT decomposes H_2O_2 into water (H_2O) and oxygen (O_2), the concentration of H_2O_2 decreases, which is reflected as a decline in absorbance. This reduction serves as an indicator of catalase activity. Observations in this study showed no significant difference between healthy and diseased plants, although CAT activity was slightly higher in healthy plants. This contrasts with findings by Dwivedi et al. (2022), where chili plants infected with Pepper leaf curl virus exhibited higher CAT activity than healthy plants. Virus-infected plants may upregulate catalase to mitigate oxidative stress caused by infection. However, some viruses can suppress catalase activity by disrupting metabolic processes or overwhelming the plant's enzymatic capacity (Amoako et al., 2015; Yang et al., 2020).

Ascorbate peroxidase (APX) is a heme-containing enzyme classified under Class I non-animal peroxidases. APX functions as both an antioxidant enzyme and a regulator of H_2O_2 signaling, and the balance of these roles is essential for managing stress responses (Maruta & Ishikawa, 2018). APX activity is indicated by a decrease in absorbance as the enzyme reduces H_2O_2 to water using ascorbate (vitamin C) as an electron donor (Maruta & Ishikawa, 2018; Rajput et al., 2021). This decrease is measurable at specific wavelengths and provides a reliable indicator of APX activity, particularly under stress conditions. APX activity in chili plants infected with yellow leaf curl disease was reported to be higher than in healthy plants (Sran et al., 2023). In this study, although no significant differences were detected between healthy and infected plants, APX activity was slightly higher in infected samples, likely due to increased demand for H_2O_2 detoxification during infection to prevent cellular oxidative damage and promote resistance.

Sampling time had a significant effect on enzyme activity in chili plants infected with PepYLCIV. The activity of defense-related enzymes changed over time following infection, indicating metabolic adaptation to viral stress. POD activity continued to increase until 28 dpi. This result is consistent with Faizah et al. (2012), who reported that peroxidase activity increased in chili genotypes infected with PepYLCIV five days (120 hours) after inoculation. CAT and APX activities fluctuated weekly throughout the observation period. Similar fluctuations in CAT activity have been reported in chili plants infected with Pepper leaf curl virus (PepLCV) by Dwivedi et al. (2022). In contrast, APX activity trends in this study differed from those reported by Hakmaoui et al. (2012), where APX activity in *Nicotiana bethamiana* infected with Pepper mild mottle virus (PMMoV) increased progressively each week.

The results also showed that CAT and APX activities were higher in fresh samples than frozen samples, whereas POD activity showed no significant difference between sample types. This finding aligns with Hartmann & Asch (2019), who observed lower APX activity in rice leaves stored at cold temperatures compared with fresh tissue. However, Lester et al. (2004) reported that freezing at −80 ºC or flash-freezing using liquid nitrogen did not significantly alter APX, CAT, or POD activity. These contrasting findings suggest that the stability of enzyme activity during frozen storage may be species-dependent. Therefore, fresh tissue is generally recommended for more accurate analysis of antioxidant enzymes, including POD, CAT, and APX. If storage is necessary, immediate freezing at −80 ºC is recommended to minimize enzymatic degradation and preserve analytical integrity (Lester et al., 2004).

Overall, enzyme activity assays in chili plants demonstrated the crucial role of POD, CAT, and APX in plant defense responses against viral infection. The results showed variations in enzyme activity depending on plant health condition, sample type, and sampling time, highlighting the complexity of biochemical responses to pathogen-induced oxidative stress. This study underscores the importance of considering these factors in enzymatic analyses to better understand plant defense dynamics.

CONCLUSION

PepYLCIV infection in chili plants significantly affected the activity of peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) enzymes. POD activity was higher in infected plants than in healthy plants, indicating a defense response to viral infection. In contrast, CAT and APX activities did not

differ significantly between infected and healthy plants ($p > 0.05$); however, both showed an increasing trend throughout the observation period. Sampling time also had a significant effect ($p < 0.05$), with POD, CAT, and APX activities increasing at specific time points. POD activity showed a significant increase at 28 dpi. CAT activity peaked at 28 dpi, although fluctuations were observed throughout the observation period. APX activity also fluctuated but reached its highest level at 5 dpi. Furthermore, sample type affected enzyme activity, with fresh samples showing higher enzyme activity than samples stored at −80 ºC.

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AUTHORS' CONTRIBUTIONS

YBP, SHH, and W designed and constructed the experiment. YBP, W, and RF performed enzyme extraction and activity assays. SI and YS carried out plant preparation, maintenance, and care after virus inoculation in the Biosafety Greenhouse. YBP and RF performed data analysis. YBP, SHH, W, KHM, and MS prepared the manuscript. All authors contributed to the research workflow, data analysis, interpretation, and manuscript revision. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that there are no competing interests, whether financial or non-financial, professional or personal, that could influence the work reported in this publication.

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