Biotic Stress Responses and Oxidative Defense Mechanisms of *Pinus brutia* against Pine Processionary Moth Infestations

Ergin Yilmaz [a,*] Esra Nurten Yer Çelik [b] Orhan Gülseven [c] Şeyma Selin Akin [d] Nezahat Turfan [e] and Sezgin Ayan [b]

* Corresponding author: yilmazergin@kastamonu.edu.tr

DOI: 10.15376/biores.20.4.9127-9147

GRAPHICAL ABSTRACT

Biotic Stress Responses and Oxidative Defense Mechanisms of *Pinus brutia* against Pine Processionary Moth Infestations

Ergin Yilmaz [a,*] Esra Nurten Yer Çelik [b] Orhan Gülseven [c] Şeyma Selin Akin [d] Nezahat Turfan [e] and Sezgin Ayan [b]

Defense mechanisms were studied for *Pinus brutia*, a cornerstone Turkish forest tree, against pine processionary moth damage by *Thaumetopoea pityocampa* (Den. & Schiff.) and *Thaumetopoea wilkinsoni* Tams 1926 moth species. This research addressed the significance of *Pinus brutia* in afforestation and breeding. The expression of enzymatic antioxidants (SOD, POD, CAT, APX) and photosynthetic pigments (chlorophylls and carotenoids) at a clonal level in response to insect damage was assessed. Approximately 84 needle samples from 28 *Pinus brutia* clones from the Antalya Düzlerçamı Brutian Pine Seed Orchard were studied. Samples were collected in February and August 2021 to capture responses during key insect activity periods. These samples were then analyzed for pigment concentrations and antioxidant activities. Statistical analysis revealed that sampling period and clone significantly affected chlorophyll and carotenoid levels. The POD and SOD activities were primarily influenced by the sampling period. However, CAT activity was affected by the number of insect pouches, the period, and the clone. APX activity was significantly impacted by both pouch number and sampling period. These findings offer insights into how seasonal changes and genetic variations modulate *P. brutia* clones' defense mechanisms against pine processionary moth infestations, informing future forest management.

DOI: 10.15376/biores.20.4.9127-9147

Keywords: Pinus brutia; Enzymatic antioxidants; Photosynthetic pigments; Clonal variation; Oxidative defense; Biotic stress; Pine processionary moth

Contact information: a: Kastamonu University, Vocational School, Department of Pharmacy Services, Kastamonu, Turkiye; b: Kastamonu University, Faculty of Forestry, Department of Silviculture, Kastamonu, Turkiye; c: Kastamonu University, Institute of Science, Kastamonu, Turkiye; d: Kastamonu University, Institute of Science, Kastamonu, Turkiye; e: Kastamonu University, Faculty of Science and Literature, Biology Department, Kastamonu, Turkiye;
** Corresponding author: yilmazergin@kastamonu.edu.tr*

INTRODUCTION

Pinus brutia Ten. is a primary forest tree species with a natural distribution in the Mediterranean and Aegean regions of Turkiye and the Eastern Aegean Islands; its wide areal range reflects high adaptation to Mediterranean climatic zones (Quezel 1979). The natural range of the species includes Crete, Cyprus, Syria, and northern Iraq, and in recent years it has been introduced into several countries with Mediterranean climates (Selik 1958; Critchfield and Little 1966; Arbez 1974; Panetsos 1981; Kara *et al*. 1997). It is tolerant of drought (Oppenheimer 1967; Nahal 1983) and is able to grow on different soil types (Quézel 1985, 2000; Milios *et al*. 2019). *Pinus brutia* is an important species for

rehabilitating degraded lands in the Mediterranean basin. As an endemic species native to the eastern Mediterranean region (Kaya and Raynal 2001), it is preferred in afforestation and reclamation efforts in Turkiye because of its rapid growth (DPT 2001). It stands out as a commercially important forest species (Usta 1990; Fady *et al*. 2003; Michelozzi *et al*. 2008).

Forest ecosystems are complex networks of interactions between trees, plants, animals, and microorganisms. Important factors threatening these ecosystems' integrity are insects and the herbivory damage that they cause (Avcı 2000). *Thaumetopoea wilkinsoni* (common in Turkiye and the Middle East) and *Thaumetopoea pityocampa* (common in Europe and North Africa) are among the most important defoliators of *Pinus* species in the Mediterranean Basin (Denis and Schiffermüller 1776; Masutti and Battisti 1990; Vega *et al.* 1997; Carus 2004; Rodríguez-Mahillo *et al*. 2012). The pine processionary moth is a widespread phytophagous species both globally and in Anatolia. It consumes the needles of *Pinus* species, an important component of Anatolian forests, leading to a decrease in the growth rates of trees (Kanat *et al*. 2005; Durkaya *et al*. 2009). It is widely distributed in warm regions of Anatolia under the influence of Mediterranean climate (Çanakçıoğlu 1993; Kanat and Türk 2002). This species, which causes significant economic losses in forest areas, can cause annual growth losses of up to 60% in *Pinus brutia*, *Pinus nigra*, and other *Pinus* species (Anonymous 1995). *Thaumetopoea* spp. larvae cause damage by feeding on the needles of *Pinus* species. While at low population densities they usually damage the twigs around their sacs, at epidemic levels they can cause defoliation and even desiccation of the trees. At later stages of larval development, the severity of damage increases in parallel with increasing nutrient requirements, reaching a maximum in the last instar larvae (Devkota and Schmidt 1990). The annual life cycle of pine processionary moth-induced defoliation negatively affects the long-term health of *Pinus* forests. Reduced annual growth of infected trees leads to physiological weakening and thus increased vulnerability to other biotic (secondary pests, pathogens) and abiotic (drought, temperature stress) stressors (Myteberi *et al*. 2013). Insect-induced herbivory triggers several biochemical processes in plant tissues that disrupt cellular homeostasis. One of these processes is the rapid and transient increase of reactive oxygen species (ROS) such as superoxide anion $O_2^{\cdot-}$ and hydrogen peroxide (H_2O_2). This ROS production represents one of the early defense responses of plant cells against damage. Increased ROS levels induce activation of the enzymatic antioxidant system, which plays an important role in plant metabolism. Superoxide dismutase (SOD) is a metalloenzyme that dismutates O_2^{-1} into H_2O_2 and molecular oxygen (O_2). Peroxidases (POD) detoxify H_2O_2 by oxidizing phenolic compounds (Skwarek *et al*. 2017). PODs are critical to plants' rapid defense mechanisms against insect damage (Gulsen *et al*. 2010; Usha Rani and Jyothsna 2010). Catalase (CAT), which has a central role in combating oxidative stress, is one of the first antioxidant enzymes discovered. The CAT catalytically cleaves H_2O_2 into water (H_2O) and O_2, thereby eliminating its toxic effect (Kerchev *et al*. 2016). The localization of CAT enzyme in different cellular compartments (mitochondria, thylakoid, and stroma of chloroplasts, cytosol and peroxisomes) and its high affinity for H_2O_2 enable it to function as an effective H_2O_2 scavenger in stressed plants and consequently play an important role in preventing cellular damage (Mushtaq *et al*. 2020). In plants, oxidative status constitutes a fundamental element of defense mechanisms against various stress factors. Rapid and transient reactive oxygen species (ROS) production is observed as a common physiological response under biotic and abiotic stress conditions (Maffei *et al*. 2007; Torres 2010). ROS, bifunctional molecules, play a role in signal transduction processes and can cause toxic effects at high

concentrations. Biotic stress-induced ROS production mechanisms and their physiological importance are among the current research topics (Maffei *et al*. 2007). The sudden and significant increase in ROS levels under stress conditions is defined as "oxidative burst" (Hare *et al*. 2011). Increases in ROS production have been found in peroxisomes, mitochondria and plasma membranes following herbivore insect damage (Maffei *et al*. 2007; Torres 2010). This ROS burst may constitute an early phase of induced defense mechanisms against pathogens and herbivores, acting as a protective barrier against subsequent attacks (Powell *et al*. 2006). Due to their high reactivity, ROS can cause oxidative damage by interacting with essential biomolecules such as proteins, lipids, and nucleic acids. To prevent this potential auto-toxicity, plant cells have evolved antioxidant defense systems that remove excess ROS and maintain ROS concentration at low and stable levels (Maffei *et al*. 2007; Howe and Jander 2008).

Temperature increases observed worldwide due to global climate change are causing a significant increase in *Thaumetopoea wilkinsoni* and *Thaumetopoea pityocampa* population densities. This increases the extent of herbivory damage to *Pinus* species (Leblebici *et al*. 2023). Considering the ecological and economic importance of *Pinus* forests worldwide and in Turkiye, it is of great importance to investigate in detail the damage caused by these defoliator species and the effects of biotic stress induced by them on oxidative stress.

Pinus brutia Ten. is one of Turkiye's important forest tree species, and breeding studies have significantly progressed. In this context, there is a need to determine different clones' resistance or sensitivity levels against pine processionary moth (*T. pityocampa* and *T. wilkinsoni*) damage. This study considered the seasonal variations of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) and enzymatic antioxidants (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX)) to determine the resistance or susceptibility of different clones in *P. brutia*, where pine processionary moth damage was intensively observed.

In this study, the resistance levels or sensitivities of *Pinus brutia* clones to pine processionary moth were evaluated. The study examined changes in the photosynthetic pigments and antioxidant enzyme levels to reveal the biological defenses of different clones against pine processionary moth and their resistance to oxidative stress. In this context, the biological responses of clones to pine processionary moth and the relationship between these responses and resistance were investigated. The basic hypotheses in the study are as follows. *Pinus brutia* clones exhibit varying levels of resistance or susceptibility to herbivore damage by *Thaumetopoea* species, depending on genotypic differences. *Thaumetopoea* damage triggers an oxidative stress response in *Pinus brutia* clones and causes a significant seasonal or interclonal effect on enzymes (SOD, POD, CAT, APX). This approach and hypotheses enabled collecting more detailed clone-based data related to pine processionary moth, which is critically important for forest management and breeding studies.

MATERIALS AND METHODS

Materials

The vegetative material of this research was obtained from the clonal seed orchard of Gölhisar provenances (*Pinus brutia* Ten.). The Brutian pine with national registration number 8, was planted in 1980 and located within the borders of Antalya Forest

Management Directorate Düzler Pine Chiefdom. This seed orchard was established with 28 different clones representing different genotypes. Within the scope of this study, needle leaf samples were collected from three genetic replicates (ramet) of each clone, recording the number of pines processionary moth pouches on the trees. Sampling was carried out during two different phenological periods in 2021: February (Period I), the dormancy period when vegetation has not started, and August (Period II), the active growth phase. The needle samples from three ramet of each clone were transferred to the Central Research Laboratory of Kastamonu University and stored at -80 °C until biochemical analyses.

Methods

All samples were collected from the uppermost lower branches of the trees' southern sides, which could be reached with pruning shears. The southern side represents an area where harmful populations may be concentrated because it receives more sunlight.

Samples were collected from pine needles during two distinct periods when damage from the pine processionary moth was either high or low.

The dependent variables examined in this study were photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) and enzymatic antioxidants (SOD, POD, CAT, and APX).

To extract and quantify photosynthetic pigments, 0.5 g of fresh needle leaf samples were taken and frozen in liquid nitrogen and powdered. The powdered samples were extracted using 10 mL of 80% acetone solution. After homogenization, the suspension was centrifuged at 3000 rpm for 10 minutes. It was centrifuged at (+4 °C). 3 mL of supernatant was used. Following centrifugation, the clear supernatant was taken and determinations were made for the amounts of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in it, spectrophotometrically (Shimadzu brand, UV Pharmaspec 1700 model, Kyoto-Japan). Absorbance values, recorded as *A* (absorbance), represent a measure of how much light is absorbed by the substance at specific wavelengths using a spectrophotometer. Absorbance values were read in a spectrophotometer at wavelengths of 450 nm (carotenoids), 645 nm (chlorophyll b), and 663 nm (chlorophyll a), respectively.

Total chlorophyll concentration was calculated using the equation described by Arnon (1949). Total carotenoid concentration was determined using a modified version of the Jaspars formula (Witham *et al*. 1971),

$$\text{Chl a} = [12.7\ (A_{663}) - 2.69\ (A_{645})]\ (V/1000 \times W) \quad (1)$$

$$\text{Chl b} = [22.9\ (A_{645}) - 4.68(A_{663})]\ (V/1000 \times W) \quad (2)$$

$$\text{Total chl a+chl b} = [20.2\ (A_{645}) + 8.02\ (A_{663})]\ (V/1000\text{x}W) \quad (3)$$

$$\text{Total carotenoid} = (4.07 \times A_{450}) - (0.0435 \times \text{chl a amount} + 0.367 \times \text{chl b amount}) \quad (4)$$

where *V* is a volume of 80% acetone, and *W* is wet weight (g) of the extracted leaf sample.

In order to determine the enzymatic antioxidant activities in the samples, 0.5 g of fresh needle leaf samples were flash frozen in liquid nitrogen and powdered. Then the obtained powder material was homogenized with 5 mL of cold extraction buffer containing 0.1 M potassium phosphate buffer (KH_2PO_4). The pH value was studied as 7. The homogenate was centrifuged at 15000 rpm for 15 min at +4 °C and obtained the supernatant. Enzyme activities were analyzed in this supernatant by spectrophotometric methods.

Catalase (CAT) activity was determined spectrophotometrically according to the protocol modified by Gong *et al*. (2001). This method monitored the rate of breakdown of hydrogen peroxide (H_2O_2) at a wavelength of 240 nm.

Superoxide dismutase (SOD) enzyme activity was determined by spectrophotometric method based on the principle of nitroblue tetrazolium (NBT) reduction inhibition applied by Agarwal and Pandey (2004). The SOD activity was calculated by measuring the amount of enzyme inhibiting NBT reduction of superoxide radicals in the reaction mixture.

Peroxidase (POD) activity was determined by the spectrophotometric method described by Yee *et al*. (2002). In this method, the increase in absorbance of the colored product formed by the oxidation of guaiacol by POD in the presence of hydrogen peroxide was monitored at 470 nm wavelength.

Ascorbate peroxidase (APX) activity was determined spectrophotometrically according to the method developed by Nakano and Asada (1981). In this method, the extent of absorbance decrease caused by the oxidation of ascorbate to dehydroascorbate by APX in the presence of hydrogen peroxide was measured at 290 nm wavelength.

Statistical Evaluation

The relationships between dependent variables (chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids, SOD, POD, CAT and APX activities) obtained from *Pinus brutia* needle leaf samples and independent variables (number of pouches, sampling period, clone and number of pouches × clone interaction) were examined by linear regression analysis using R statistical software.

Analysis of Variance (ANOVA) was applied to determine the main and interaction effects of independent factors (clone, number of pouches, period and clone × number of pouches) on the variables analyzed.

Duncan Multiple Comparison Test was used to determine homogeneous groups and to make multiple comparisons between means in variables showing significant differences according to ANOVA results. Significance level was accepted as $P < 0.05$ in statistical analyses.

RESULTS AND DISCUSSION

The results of statistical analysis between enzymatic antioxidant activities (SOD, POD, CAT, APX) and independent variables (number of pouches, sampling period, clone and pouch number × clone interaction) are presented in Table 1.

The data presented in Table 1 show that enzymatic antioxidant activities (SOD, POD, CAT, APX) were highest in February, when intense biotic stress from pine processionary moth (*Thaumetopoea* spp.) damage was observed. However, these activities decreased significantly in August, when damage decreased.

This finding suggests that plants combat oxidative stress by activating their enzymatic antioxidant systems against pine processionary moth attack, and that these defense mechanisms revert to their previous state when the stress load decreases.

Similarly, the results of statistical analysis between photosynthetic pigment concentrations (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) and the same independent variables are summarized in Table 2.

Table 1. Evaluation of Enzymatic Antioxidant Activities by Linear Regression Analysis

Dependent Variable	Constant Value (β_0)		Independent Variable					
			Number of Pouches		Period		Clone	
	F	P	Forecast	P	Forecast	P	F	P
APX (EU mg/protein)	390.6503	*<0.0001*	0.0019703	*0.0121*	-0.016797	*0.0043*	1.2135	*ns*
CAT (EU mg/protein)	1158.4894	*<0.0001*	0.00703	*0.0067*	-0.08553	*0.0001*	1.8086	*0.0315*
POD (EU mg/protein)	90.8301	*<0.0001*	-0.011845	*ns*	-0.75062	*0.0001*	0.8884	*ns*
SOD (EU mg/protein)	69.43603	*<0.0001*	-3.266	*ns*	-73.925	*0.0001*	0.89163	*ns*

The seasonal effect of pine processionary moth damage on photosynthetic pigments is a significant finding. Chlorophyll *a*, *b*, and total chlorophyll amounts decreased across the sampling period, but the amount of chlorophyll and interactions among clones had limited effects on the pigments.

Table 2. Evaluation of Photosynthetic Pigment Levels with Linear Regression Equations

Dependent Variable	Constant Value (β_0)		Independent Variable			
			Period		Clone	
	F	P	Forecast	P	F	P
Chlorophyll *a* (mg/g)	5934.797	*<0.0001*	-0.016080	*0.0001*	2.252	0.0052
Chlorophyll *b* (mg/g)	2715.977	*<0.0001*	-0.0164283	*0.0001*	1.3667	ns
Total Chlorophyll (mg/g)	5181.577	*<0.0001*	-0.03248	*0.0001*	1.957	0.0172
Carotenoid (mg/g)	6111.8	*<0.0001*	-0.77481	*0.0001*	1.684	0.0503

The effects of independent variables (number of pouches, sampling period, clone and number of pouches × clone interaction) on APX, CAT, POD, and SOD enzyme activities were analyzed. According to the results of linear regression analysis, the significant effects of pouch number and sampling period on APX activity were determined ($P < 0.05$). In contrast, the effects of clone and pouch number × clone interaction were not statistically significant ($P > 0.05$). While APX activity levels decreased from February to August, increased APX activity was observed with increased pouches.

CAT activity was significantly affected by the number of pouches, sampling period and clone factors ($P < 0.05$), but the effect of pouch number × clone interaction was not significant ($P > 0.05$). CAT activity also tended to decrease periodically, while an increase in CAT activity was detected with the increase in pouches.

For POD activity, the sampling period factor was found to be significant ($P < 0.05$); the effect of other factors was not statistically significant ($P > 0.05$). The POD activity levels decreased with the transition from February to August. Similarly, only the sampling period had a significant effect on SOD activity ($P < 0.05$), while the effect of other factors was not statistically significant ($P > 0.05$). The SOD activity levels also showed a periodic decrease from February to August.

According to the results of analysis of variance (ANOVA) and Duncan's Multiple Comparison Test, we present the homogeneous groups of sampling periods (February and August) for SOD, POD, CAT, and APX enzyme activities in Table 3.

Table 3. Variation of Enzymatic Antioxidant (SOD, POD, CAT, APX) Activities in Different *Pinus brutia* Clones in I- (February) and II- (August) Periods

PERIOD I				PERIOD II			
SOD Enzyme Activity (EU/mg protein)							
Clone No.	**Pouches No.**	**X±Sx**	**Groups**	**Clone No.**	**Pouches No.**	**X±Sx**	**Groups**
8586	7	43,85±1,36	d	8570	8	19,12±2,20	f
8582	4	44,23±13,01	d	8571	8	21,46±1,41	ef
8562	8	45,03±5,23	d	8569	10	22,66±3,27	def
8573	8	48,43±6,54	d	8574	4	25,36±2,74	def
8572	3	52,64±3,13	d	8581	4	28,96±3,40	def
8583	6	53,82±8,30	d	8585	4	29,42±5,46	def
8581	4	54,46±8,17	d	8575	4	33,62±4,88	def
8569	10	55,84±12,12	d	8580	5	34,67±3,44	def
8579	4	59,87±4,20	d	8578	6	37,94±5,09	cdef
8563	4	61,84±8,36	d	8587	5	38,67±4,20	cdef
8565	3	63,87±12,45	d	8561	6	38,82±10,45	cdef
8564	5	78,47±15,85	d	8565	3	40,96±4,99	cdef
8570	8	81,44±11,43	d	8562	8	41,70±8,29	cdef
8571	8	82,81±5,88	d	8563	4	42,22±6,47	bcdef
8580	5	84,35±15,14	d	8583	6	42,90±12,01	bcdef
8578	6	92,45±9,57	d	8566	3	43,15±6,01	bcdef
8587	5	104,22±11,35	cd	8576	3	46,75±3,77	bcdef
8576	3	115,68±21,78	cd	8586	7	51,70±4,51	bcdef
8567	4	117,40±25,05	cd	8577	6	51,81±8,11	bcdef
8577	6	117,70±18,39	cd	8564	5	54,00±13,98	bcdef
8560	4	123,22±32,28	cd	8579	4	54,48±6,55	bcdef
8584	4	123,61±9,25	cd	8567	4	54,78±5,68	bcdef
8561	6	170,77±67,72	cd	8582	4	58,12±19,74	bcdef
8574	4	179,51±38,48	cd	8584	4	59,27±5,58	bcde
8575	4	186,36±69,22	cd	8560	4	61,67±9,46	bcd
8568	4	270,18±79,24	b	8572	3	75,71±21,16	abc
8566	3	407,71±170,49	ab	8568	4	80,82±11,20	ab
8585	4	444,63±162,07	a	8573	8	103,72±40,63	a
F-value	3,758			F-value	2,724		
P-level	0,000			P-level	0,000		
PERIOD I				**PERIOD II**			
POD Enzyme Activity (EU/mg protein)							
Clone No.	**Pouches No.**	**X±Sx**	**Groups**	**Clone No.**	**Pouches No.**	**X±Sx**	**Groups**
8586	7	0,16±0,01	h	8563	4	0,001± 0,0003	h
8580	5	0,21±0,01	gh	8580	5	0,001± 0,0001	h
8583	6	0,21±0,01	gh	8583	6	0,002± 0,0009	gh
8572	3	0,23±0,04	gh	8578	6	0,003± 0,0005	gh
8562	8	0,23±0,03	gh	8577	6	0,003± 0,0005	gh
8569	10	0,29±0,04	fgh	8571	8	0,003± 0,0008	fgh
8565	3	0,33±0,06	efgh	8562	8	0,003± 0,0002	fgh
8571	8	0,48±0,13	defgh	8575	4	0,004± 0,0009	efgh
8579	4	0,52±0,15	cdefgh	8569	10	0,004± 0,0005	defgh
8581	4	0,59±0,19	cdefgh	8568	4	0,004± 0,0012	defgh
8564	5	0,61±0,08	cdefgh	8587	5	0,005± 0,0011	defgh
8573	8	0,66±0,09	cdefgh	8565	3	0,005± 0,0002	defgh
8561	6	0,69±0,17	bcdefgh	8584	4	0,005± 0,0011	
8577	6	0,71±0,18	bcdefgh	8579	4	0,005± 0,0005	defgh
8576	3	0,74±0,16	bcdefgh	8570	8	0,005± 0,0006	defgh
8585	4	0,83±0,22	bcdefgh	8586	7	0,006± 0,0014	cdefg
8570	8	0,87±0,36	abcdefgh	8581	4	0,006± 0,0007	cdefg
8566	3	0,90±0,14	abcdefgh	8576	3	0,006± 0,0011	cdefg
8563	4	0,92±0,24	abcdefgh	8574	4	0,007± 0,0024	bcdefg

8578	6	0,93±0,21	abcdefgh	8585	4	0,008± 0,0021	abcdefg
8560	4	0,98±0,30	abcdefg	8582	4	0,009± 0,0017	abcdefg
8567	4	1,09±0,34	abcdef	8564	5	0,010± 0,0006	abcdef
8568	4	1,11±0,40	abcde	8566	3	0,011± 0,0037	abcde
8575	4	1,15±0,20	abcd	8561	6	0,011± 0,0031	abcd
8582	4	1,22±0,51	abcd	8560	4	0,012± 0,0020	abc
8584	4	1,31±0,27	abc	8572	3	0,013± 0,0033	ab
8574	4	1,48±0,06	ab	8573	8	0,015± 0,0056	a
8587	5	1,63±0,52	a	8567	4	0,015± 0,0047	a
F-value	2,997			F-value	3,690		
P-level	0,000			P-level	0,000		
PERIOD I				**PERIOD II**			
CAT Enzyme Activity (EU/mg protein)							
Clone No.	**Pouches No.**	**X±Sx**	**Groups**	**Clone No.**	**Pouches No.**	**X±Sx**	**Groups**
8574	4	0,20±0,030	g	8560	4	0,17±0,014	e
8584	4	0,21±0,005	fg	8567	4	0,22±0,011	de
8587	5	0,21±0,024	fg	8565	3	0,23±0,010	cde
8568	4	0,22±0,057	fg	8577	6	0,23±0,018	cde
8566	3	0,22±0,036	fg	8586	7	0,23±0,019	cde
8578	6	0,24±0,019	fg	8566	3	0,24±0,005	cde
8575	4	0,28±0,034	efg	8576	3	0,25±0,020	cde
8560	4	0,31±0,038	defg	8572	3	0,26±0,072	bcde
8576	3	0,31±0,063	defg	8582	4	0,26±0,054	bcde
8585	4	0,32±0,028	defg	8584	4	0,27±0,050	bcde
8567	4	0,34±0,037	cdef	8578	6	0,27±0,041	bcde
8577	6	0,34±0,062	cdef	8564	5	0,28±0,021	bcde
8571	8	0,34±0,029	cdef	8580	5	0,28±0,014	bcde
8564	5	0,35±0,020	cdef	8583	6	0,29±0,021	bcde
8579	4	0,38±0,007	cde	8585	4	0,29±0,032	bcde
8583	6	0,40±0,030	cde	8570	8	0,30±0,035	bcde
8570	8	0,41±0,043	cde	8579	4	0,30±0,044	abcde
8561	6	0,41±0,040	cde	8581	4	0,31±0,024	abcde
8569	10	0,44±0,049	cd	8568	4	0,32±0,096	abcde
8562	8	0,44±0,064	cd	8563	4	0,32±0,004	abcde
8563	4	0,44±0,057	cd	8561	6	0,33±0,038	abcd
8573	8	0,45±0,030	cd	8562	8	0,34±0,028	abcd
8572	3	0,47±0,046	bc	8573	8	0,34±0,084	abcd
8565	3	0,48±0,017	bc	8574	4	0,38±0,029	abc
8582	4	0,60±0,098	ab	8587	5	0,38±0,086	abc
8586	7	0,61±0,017	a	8575	4	0,40±0,074	ab
8581	4	0,62±0,007	a	8571	8	0,44±0,025	a
8580	5	0,72±0,019	a	8569	10	0,45±0,004	a
F-value	10,560			F-value	2,332		
P-level	0,000			P-level	0,000		
PERIOD I				**PERIOD II**			
APX Enzyme Activity (EU/mg protein)							
Clone No.	**Pouches No.**	**X±Sx**	**Groups**	**Clone No.**	**Pouches No.**	**X±Sx**	**Groups**
8566	3	0,017±0,002	f	8560	4	0,030±0,004	d
8583	6	0,027±0,011	ef	8586	7	0,043±0,002	cd
8577	6	0,032±0,009	def	8584	4	0,046±0,008	bcd
8578	6	0,032±0,006	def	8577	6	0,059±0,012	abcd
8582	4	0,032±0,007	def	8579	4	0,060±0,008	abcd
8574	4	0,036±0,01	def	8562	8	0,060±0,012	abcd
8567	4	0,041±0,01	def	8583	6	0,062±0,008	abcd
8568	4	0,044±0,007	def	8566	3	0,063±0,009	abcd
8587	5	0,60±0,018	cdef	8570	8	0,063±0,008	abcd
8585	4	0,071±0,01	bcdef	8564	5	0,071±0,008	abcd
8572	3	0,073±0,01	bcdef	8580	5	0,072±0,012	abcd

8575	4	0,081±0,03	bcdef	8563	4	0,073±0,009	abcd
8561	6	0,082±0,01	bcdef	8565	3	0,073±0,007	abcd
8565	3	0,091±0,03	bcdef	8578	6	0,074±0,018	abcd
8584	4	0,094±0,02	abcdef	8576	3	0,075±0,006	abcd
8576	3	0,096±0,03	abcdef	8574	4	0,075±0,006	abcd
8571	8	0,097±0,01	abcdef	8569	10	0,078±0,014	abcd
8569	10	0,103±0,01	abcdef	8585	4	0,078±0,016	abcd
8586	7	0,107±0,03	abcdef	8561	6	0,079±0,013	abcd
8560	4	0,112±0,03	abcde	8572	3	0,079±0,021	abcd
8570	8	0,120±0,02	abcde	8582	4	0,079±0,020	abcd
8564	5	0,124±0,02	abcd	8573	8	0,079±0,031	abcd
8579	4	0,124±0,03	abcd	8567	4	0,080±0,009	abcd
8563	4	0,139±0,02	abc	8575	4	0,082±0,018	abcd
8573	8	0,139±0,04	abc	8568	4	0,086±0,025	abc
8581	4	0,156±0,02	ab	8581	4	0,088±0,020	abc
8580	5	0,164±0,04	ab	8587	5	0,098±0,033	ab
8562	8	0,184±0,05	a	8571	8	0,104±0,013	a
F-value	2,821			F-value	1,000		
P-level	0,000			P-level	0,470		

Significant effects of sampling period and clone factors on chlorophyll-a (cl-a) levels were determined ($P < 0.05$), whereas the effects of pouch number and pouch number × clone interaction were not statistically significant ($P > 0.05$). Chlorophyll-a concentration showed a seasonal decrease from February to August. For chlorophyll-b (kl-b) levels, only the sampling period factor was significant ($P < 0.05$), the effect of other factors was not statistically significant ($P > 0.05$). Chlorophyll-b concentrations similarly decreased with the transition from February to August. Total chlorophyll content was also significantly affected by clone and sampling period factors. However, the effect of the number of sacs and the sac number × clone interaction was not statistically significant ($P > 0.05$). Total chlorophyll content showed a decrease from February to August. When the relationships between carotenoid concentrations and independent variables (number of pouches, sampling period, clone and number of pouches × clone interaction) were analyzed, it was determined that the sampling period and clone factors had statistically significant effects on carotenoid content ($P < 0.05$). On the other hand, sac number and sac number × clone interaction had no statistically significant effect on carotenoid content ($P > 0.05$) (Table 2). When the seasonal variation was analyzed, it was observed that carotenoid content decreased significantly in August compared to February. According to the analysis of variance (ANOVA) and Duncan's Multiple Comparison Test, homogeneous groups for the sampling periods (February and August) for chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid amounts are presented in Table 4.

Table 4. Variation in Photosynthetic Pigment (Chlorophyll-a, Chlorophyll-b, Total Chlorophyll, and Carotenoids) Concentrations in Different *Pinus brutia* Clones in I- (February) and II- (August) Periods

PERIOD I				PERIOD II			
Chlorophyll A (mg/g)							
Clone No.	**Pouches No.**	**X±Sx**	**Groups**	**Clone No.**	**Pouches No.**	**X±Sx**	**Groups**
8583	6	0,05±0,005	e	8575	4	0,0386±0,003	I
8582	4	0,05±0,006	e	8563	4	0,0396±0,002	ii
8564	5	0,05±0,004	e	8587	5	0,0431±0,002	hıi
8572	3	0,05±0,005	de	8564	5	0,0451±0,006	ghıi

8560	4	0,06±0,005	cde	8576	3	0,0455±0,003	ghıi
8562	8	0,06±0,005	cde	8585	4	0,0481±0,003	fghıi
8587	5	0,06±0,005	cde	8565	3	0,0488±0,003	efghı
8586	7	0,06±0,005	cde	8581	4	0,0489±0,002	efghı
8579	4	0,06±0,006	bcde	8572	3	0,0514±0,001	defgh
8570	8	0,06±0,0055	bcde	8583	6	0,0515±0,005	defgh
8584	4	0,06±0,006	bcde	8579	4	0,0526±0,00059	cdefg
8565	3	0,06±0,007	abcde	8582	4	0,053±0,002	cdefg
8561	6	0,06±0,007	abcde	8567	4	0,0532±0,005	cdefg
8563	4	0,06±0,005	abcde	8571	8	0,0549±0,003	bcdefg
8580	5	0,06±0,006	abcde	8568	4	0,0563±0,004	bcdef
8578	6	0,07±0,006	abcde	8586	7	0,0564±0,003	bcdef
8575	4	0,07±0,007	abcde	8560	4	0,0568±0,0007	bcdef
8576	3	0,07±0,004	abcde	8580	5	0,0572±0,003	bcdef
8581	4	0,07±0,006	abcde	8574	4	0,0579±0,005	bcdef
8566	3	0,07±0,003	abcde	8562	8	0,0581±0,002	bcdef
8571	8	0,07±0,008	abcde	8569	10	0,0594±0,001	bcde
8577	6	0,07±0,005	abcde	8577	6	0,06±0,0041	abcd
8568	4	0,07±0,007	abcd	8570	8	0,0603±0,003	abcd
8573	8	0,07±0,005	abcd	8566	3	0,0608±0,002	abcd
8585	4	0,08±0,006	abc	8584	4	0,615±0,0057	abcd
8574	4	0,08±0,006	abc	8578	6	0,6251±0,004	abc
8567	4	0,08±0,006	ab	8561	6	0,0652±0,0009	ab
8569	10	0,08±0,007	a	8573	8	0,0701±0,001	a
F-value	2,025			F-value	5,831		
P-level	0,003			P-level	0,000		
PERIOD I				**PERIOD II**			
Chlorophyll B (mg/g)							
Clone No.	**Pouches No.**	**X±Sx**	**Groups**	**Clone No.**	**Pouches No.**	**X±Sx**	**Groups**
8564	5	0,03±0,003	e	8563	4	0,0174±0,0013	h
8583	6	0,03±0,002	e	8576	3	0,0195±0,0011	gh
8582	4	0,03±0,004	de	8564	5	0,0204±0,0032	fgh
8572	3	0,03±0,003	de	8587	5	0,0205±0,0014	fgh
8562	8	0,03±0,003	cde	8574	4	0,0217±0,0061	efgh
8570	8	0,03±0,004	cde	8567	4	0,0202±0,0035	defgh
8575	4	0,03±0,004	cde	8581	4	0,0223±0,0009	cdefgh
8584	4	0,03±0,005	cde	8583	6	0,0224±0,0023	cdefgh
8565	3	0,03±0,003	bcde	8582	4	0,0225±0,0007	bcdefgh
8579	4	0,03±0,005	bcde	8585	4	0,0227±0,0015	bcdefgh
8581	4	0,03±0,002	bcde	8572	3	0,0228±0,0014	bcdefgh
8560	4	0,03±0,004	bcde	8579	4	0,0244±0,001	abcdefgh
8563	4	0,04±0,004	abcde	8580	5	0,0247±0,0008	abcdefg
8587	5	0,04±0,003	abcde	8569	10	0,0252±0,0002	abcdefg
8576	3	0,04±0,005	abcde	8560	4	0,0256±0,0006	abcdefg
8580	5	0,04±0,003	abcde	8568	4	0,0257±0,0013	abcdefg
8561	6	0,04±0,004	abcde	8575	4	0,0263±0,0027	abcdefg
8571	8	0,04±0,004	abcde	8586	7	0,0268±0,001	abcdefg
8566	3	0,04±0,004	abcde	8571	8	0,0269±0,0006	abcdefg
8578	6	0,04±0,003	abcde	8584	4	0,0272±0,0028	abcdef
8577	6	0,04±0,004	abcde	8565	3	0,0273±0,0039	afbcdef
8573	8	0,04±0,004	abcde	8577	6	0,0275±0,0021	abcde
8585	4	0,04±0,003	abcde	8570	8	0,0288±0,00085	abcde
8574	4	0,04±0,003	abcd	8561	6	0,0292±0,0005	abcd
8568	4	0,04±0,004	abcd	8562	8	0,096±0,0017	abc
8586	7	0,05±0,004	abc	8578	6	0,0299±0,0013	ab
8569	10	0,05±0,006	ab	8566	3	0,0304±0,0015	a
8567	4	0,05±0,007	a	8573	8	0,0308±0,0014	a
F-value	2,777			F-value	1,908		
P-level	0,000			P-level	0,006		

PERIOD I				PERIOD II			
Total Chlorophyll (mg/g)							
Clone No.	Pouches No.	X±Sx	Groups	Clone No.	Pouches No.	X±Sx	Groups
8583	6	0,08±0,007	f	8563	4	0,0569±0,0034	i
8564	5	0,08±0,007	ef	8587	5	0,0636±0,0037	ıi
8582	4	0,08±0,010	def	8575	4	0,0649±0,0014	ıi
8572	3	0,09±0,008	cdef	8576	3	0,065±0,0039	ıi
8562	8	0,10±0,007	cdef	8564	5	0,0655±0,003	hıi
8560	4	0,10±0,010	cdef	8585	4	0,0709±0,0043	ghıi
8570	8	0,10±0,009	cdef	8581	4	0,0712±0,0029	fghıi
8584	4	0,10±0,010	cdef	8583	6	0,0739±0,0068	efghı
8579	4	0,10±0,011	cdef	8572	3	0,0742±0,0025	efghı
8587	5	0,10±0,008	cdef	8567	4	0,0752±0,0081	defghı
8565	3	0,10±0,010	cdef	8582	4	0,0755±0,0027	defghı
8575	4	0,10±0,010	bcdef	8565	3	0,0761±0,0066	cdefghı
8563	4	0,11±0,009	abcdef	8579	4	0,0769±0,0015	cdefghı
8561	6	0,11±0,011	abcdef	8574	4	0,0795±0,0095	bcdefghı
8580	5	0,11±0,008	abcdef	8571	8	0,0818±0,0038	bcdefgh
8581	4	0,11±0,008	abcdef	8580	5	0,0819±0,0032	bcdefgh
8576	3	0,11±0,009	abcdef	8568	4	0,082±0,0051	bcdefgh
8586	7	0,11±0,009	abcdef	8560	4	0,0824±0,0012	bcdefg
8578	6	0,11±0,009	abcdef	8586	7	0,0832±0,0039	bcdefg
8571	8	0,11±0,011	abcdef	8569	10	0,0846±0,0009	bcdefg
8566	3	0,11±0,006	abcde	8577	6	0,0875±0,0062	abcdefg
8577	6	0,12±0,008	abcd	8562	8	0,0878±0,0039	abcdef
8573	8	0,12±0,009	abc	8584	4	0,0888±0,0085	abcde
8568	4	0,12±0,011	abc	8570	8	0,0891±0,0037	abcde
8585	4	0,12±0,009	abc	8566	3	0,0912±0,0031	abcd
8574	4	0,12±0,008	abc	8578	6	0,0924±0,0053	abc
8569	10	0,14±0,012	ab	8561	6	0,0947±0,0014	ab
8567	4	0,14±0,010	a	8573	8	0,103±0,0029	a
F-value P-level	2,163 0,001			F-value P-level	4,5957 0,000		
PERIOD I				PERIOD II			
Carotenoid (mg/g)							
Clone No.	Pouches No.	X±Sx	Groups	Clone No.	Pouches No.	X±Sx	Groups
8582	4	5,75±0,45	h	8563	4	4,76±0,25	h
8564	5	5,84±0,35	gh	8576	3	4,9±0,27	gh
8583	6	5,96±0,29	efgh	8564	5	5,44±0,7	fgh
8560	4	6,19±0,31	defgh	8565	3	5,49±0,24	efgh
8586	7	6,31±0,41	defgh	8587	5	5,51±0,34	defgh
8572	3	6,36±0,38	defgh	8583	6	5,57±0,50	defgh
8570	8	6,39±0,42	defgh	8582	4	5,75±0,38	cdefgh
8587	5	6,40±0,39	defgh	8581	4	5,76±0,09	cdefgh
8580	5	6,44±0,46	cdefgh	8567	4	5,85±0,71	cdefgh
8562	8	6,74±0,38	bcdefgh	8575	4	5,97±0,06	cdefgh
8579	4	6,82±0,52	bcdefgh	8572	3	5,99±0,33	cdefgh
8584	4	6,87±0,56	abcdefgh	8574	4	5,99±0,86	cdefgh
8563	4	6,99±0,47	abcdefgh	8580	5	6,06±0,15	cdefg
8561	6	7,03±0,66	abcdefgh	8579	4	6,27±0,05	cdef
8576	3	7,03±0,32	abcdefgh	8585	4	6,29±0,42	cdef
8565	3	7,04±0,56	abcdefgh	8584	4	6,36±0,56	bcdef
8581	4	7,09±0,36	abcdefgh	8571	8	6,4±0,06	abcdef
8575	4	7,12±0,52	abcdefgh	8568	4	6,41±0,15	abcdef
8566	3	7,34±0,35	abcdefg	8560	4	6,63±0,15	abcdef
8577	6	7,44±0,23	abcdef	8570	8	6,67±0,18	abcdef
8571	8	7,46±0,54	abcdef	8577	6	6,71±0,46	abcdef

8568	4	7,67±0,54	abcde	8586	7	6,74±0,13	abcde
8578	6	7,72±0,42	abcde	8569	10	6,8±0,02	abcd
8567	4	7,75±0,55	acbd	8578	6	6,9±0,32	abc
8573	8	7,97±0,48	abc	8561	6	7,008±0,18	abc
8585	4	8,00±0,45	ab	8562	8	7,02±0,26	abc
8574	4	8,07±0,38	ab	8573	8	7,58±0,35	ab
8569	10	8,39±0,34	a	8566	3	7,64±0,36	a
F-value	2,555			F-value	3,580		
P-level	0,000			P-level	0,000		

DISCUSSION

The findings of this study showed that photosynthetic pigment concentrations (chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoids) in *Pinus brutia* needle leaves were significantly affected not only by abiotic environmental factors but also by biotic stress factors caused by the pine processionary moths (*Thaumetopoea pityocampa* and *Thaumetopoea wilkinsoni*). In particular, an increase in photosynthetic pigment levels was observed in February, the active feeding period of pine processionary moth larvae. *Thaumetopoea* spp. cause defoliation of *P. brutia* individuals through their feeding activities in winter and early spring (intensively in February-March). This defoliation is an important biotic stress factor that can decrease tree growth performance and mortality in young plantations in cases of severe infection (Carus 2004; Battisti *et al*. 2005; Kanat *et al*. 2005).

The results of this study revealed that chlorophyll-a (chl-a) concentration in *Pinus brutia* needles was significantly affected by both sampling period and clone factors. In contrast, chlorophyll-b (cl-b) concentration was significantly affected only by sampling period factor. Chlorophyll levels were found to be significantly higher in the first sampling period (February), when the impact of pine processionary moth (*Thaumetopoea* spp.) was particularly intense, compared to the second period (August). The highest chl-a (0.08 mg/g wet weight) and total chlorophyll (0.14 mg/g wet weight) contents in clone N8569 (10 pouches), which had the highest number of pouches in the same period, support the hypothesis that biotic stress caused by pine processionary moth may induce pigment biosynthesis as a defense mechanism in plants. These findings are in agreement with the literature that plants use pigment production as an adaptation strategy to optimize their photosynthetic capacity under stress conditions. For example, Tanaka and Tanaka (2011) reported that chlorophyll-a and chlorophyll-b pigments can interconvert in response to environmental stresses. This dynamic conversion is a physiological adaptation mechanism to exogenous stress signals. Similarly, Nouri *et al*. (2023) emphasized that genotypes tolerant to stress conditions generally have higher chlorophyll and carotenoid contents, which increases the overall resilience of plants against biotic and abiotic stresses. In this context, the high pigment levels observed in individuals with high sac counts in the present study can be interpreted as a physiological response to biotic damage.

Changes in photosynthetic pigment concentrations between February and August also reflect the significant effects of abiotic environmental factors. Sauceda *et al*. (2008) reported that the observed variations in chlorophyll content were closely related to abiotic stress factors such as water stress and high light intensity. Increased temperature and light intensity in summer can inhibit the biosynthesis of photosynthetic pigments, leading to a decrease in chlorophyll and carotenoid levels (Yordanov *et al*. 2000; Pukacki and Kamińska-Rożek 2005). In this study, a significant decrease in chlorophyll and carotenoid

levels was generally detected in August compared to February (Table 2, Table 4). Brett and Singer (1973) also stated that high light and temperature conditions may decrease chlorophyll content. However, it can be concluded that this seasonal variation in this study is largely due to environmental factors and that the damage by the pine processionary moth (*Thaumetopoea* spp.) has an increasing effect on pigment biosynthesis. Therefore, it is thought that significant differences emerged between the sampling periods and pigment concentrations obtained in the first period (February) were higher than in the second period (August).

A similar trend was observed for carotenoid concentrations. Statistical analyses revealed that sampling period and clone factors significantly affected carotenoid levels. Carotenoid levels were significantly higher in February compared to August. Carotenoids are important antioxidant molecules in protecting chlorophyll against photooxidative damage and detoxification of reactive oxygen species (ROS), as well as functioning as auxiliary pigments in the photosynthetic antenna system (Zhang *et al*. 2021). These properties play a critical role in the defense mechanisms of plants against biotic stress factors such as pine processionary moth (*Thaumetopoea* spp.). Nouri *et al*. (2023) also provided evidence supporting these findings, stating that genotypes tolerant to stress conditions generally have higher levels of carotenoids.

The fact that both chlorophyll and carotenoid concentrations were found to be high in February, when pine processionary moth (*Thaumetopoea* spp.) damage was evident, suggests that biotic stress has an up-regulating effect on pigment biosynthesis in *Pinus brutia* individuals. This may be considered as an important component of the defense mechanisms developed by the plant against herbivory. The observed variability in photosynthetic pigment levels as a result of synergistic or antagonistic interactions of biotic and abiotic stressors is critical for developing a deeper understanding of the complex stress physiology of plants.

As a result of examining the relationships between enzymatic antioxidants (SOD, POD, CAT, and APX) and pine processionary moth pouch number, sampling period, clone and pouch number × clone interactions, a significant positive correlation was found between pouch number and sampling period on APX activity. The CAT activity was significantly affected by the number of pouches, sampling period and clone factors, while SOD and POD activities were significantly correlated only with the sampling period factor (Table 1). Literature reviews show limited studies on enzymatic antioxidant responses in *Pinus brutia*. Plants increase their survival probability by activating defense mechanisms against biotic stressors such as herbivorous insects. One of these defense mechanisms is the increased activity of enzymatic antioxidant systems triggered by the production of reactive oxygen species (ROS). Superoxide dismutase dismutates the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2), increasing the tolerance of plants to oxidative stress, while the POD catalyzes the oxidation of phenolic compounds using H_2O_2 (Katyshev *et al*. 2006; Boguszewska *et al*. 2010). These antioxidant enzymes protect against potential damage caused by oxidative damage in plant cells (Hashemi 2019).

Biotic stressors such as herbivorous insects enhance defense mechanisms against oxidative stress by increasing the activities of SOD, POD, CAT, and APX in plants. These enzymatic responses play an important role in enhancing the physiological responses of plants to biotic stress and thus their survival capacity (Xu *et al*. 2015). In this study, a significant increase in enzymatic antioxidant activities such as SOD, POD, CAT, and APX was observed in February when pine processionary moth (*Thaumetopoea* spp.) damage was effective. Skwarek *et al*. (2017) reported differences in enzymatic antioxidant levels

between species due to *Melolontha melolontha* causing root damage in *Pinus sylvestris* and *Larix decidua* species. This finding in the present study suggests that *Pinus brutia* individuals are more susceptible to pine processionary moth-induced biotic stress in February and therefore activate their defense mechanisms more intensively. In August, a decrease in these enzymatic activities was observed with the decrease in pine processionary moth damage.

The results of the analysis revealed that all enzymatic antioxidant activities (SOD, POD, CAT, APX) showed a significant decrease from February, when pine processionary moth (*Thaumetopoea* spp.) damage was intense, to August, when the processionary moth effect decreased (Table 1). This finding indicates that antioxidant enzymes play a more active role against oxidative damage during the period of high biotic stress and that the activity of these enzymatic defense mechanisms decreases during the period of reduced stress. Thus, this study clearly demonstrates that a specific biotic stressor such as pine processionary moth dynamically affects the enzymatic antioxidant activities of *Pinus brutia* individuals, triggering their defense response and that this defense response shows seasonal changes.

The results from this study revealed that CAT enzyme activity was significantly correlated with pine processionary moth pouch number, sampling period and *Pinus brutia* clone (Table 1). The plant plasma membrane constantly interacts with the external environment, which can activate signal transduction pathways. Biotic and abiotic stress factors can modulate ion flow by causing abrupt changes in cell membrane potential (Ebel and Mithöfer 1998; Shabala 2006). Damage signals caused by herbivorous insects can lead to generating electrical signals that propagate throughout the plant (Maffei and Bossi 2006). Hydrogen peroxide can be strongly depolarized by insect feeding (Peiffer and Felton 2005). In addition to mechanical damage, plants can recognize herbivore-specific elicitor molecules. These elicitors can be found in insect oral secretions (Halitschke *et al*. 2001), oviposition secretions (Voirol *et al*. 2020), and feces (frass) (Ray *et al*. 2015). In a study by Liu *et al*. (2019), bark processionary moths did not alter POD activity on *Pinus yunnanensis* but increased CAT activity. CAT plays an important role in meeting the increased energy demand of the plant under stress conditions by removing H_2O_2 (Kerchev *et al*. 2016). Moreover, H_2O_2 induced by salicylic acid can damage the digestive system of insects and inhibit their growth and development (Peng *et al*. 2004; Maffei *et al*. 2007). These literature findings support the significant relationship of CAT enzyme with the present study's findings for the number of pouches, sampling period, and clone. Skwarek *et al*. (2017) reported that insect damage increased the activities of SOD and POD enzymes. Liu *et al*. (2019) observed an increase in the levels of SOD, POD, and CAT enzymes as a result of *Tomicus yunnanensis* Kirkendall and Faccoli and *Tomicus* minor Hartwig damage in their study on *Pinus yunnanensis* Franch.

The results obtained in this study showed that only the sampling period factor was statistically significant in the relationship between SOD enzyme activity and pine processionary moth pouch number, sampling period, clone and pouch number × clone interaction (Table 1). The SOD enzyme provides a protective mechanism against cellular oxidative damage by converting superoxide radical (O_2-) to hydrogen peroxide (H_2O_2), and this process plays a critical role in the defense responses of plants against biotic and abiotic stresses (Jabs *et al*. 1997). Furthermore, the enzymes SOD, POD, CAT, and APX detoxify $O_2-$and H_2O_2, forming a synergistic protection mechanism against these stresses (Mittler 2002; Prattipati *et al*. 2021). The POD enzymes are an important group of enzymes that rapidly activate plant defense responses against insect damage and can inhibit insect

growth by oxidizing phenolic compounds (War *et al*. 2012). Liu *et al*. (2019) observed an increase in SOD, POD, and CAT activities after *Tomicus yunnanensis* and *Tomicus* minor damage. Skwarek *et al*. (2017) found that insect damage on *Pinus sylvestris* and *Larix decidua* increased SOD enzyme activities. These literature findings support that biotic stress leads to the induction of enzymatic responses that enhance plant defense (Lamb and Dixon 1997; Keeling and Bohlmann 2006).

The pine processionary moth directly damages the tree and can trigger biological defense systems, leading to more subtle weakening. The insect's feeding behaviors, particularly chemical salivary secretions, can increase the tree's oxidative stress levels and trigger biological responses. Such indirect effects can affect tree health long-term but may not be detectable through direct observation. Therefore, a complete understanding of the pest's effects requires considering visible damage and the tree's biological responses. Furthermore, trees employ tolerance to herbivore attacks, which is the ability to maintain their fitness despite damaged tissue. This tolerance encompasses both visible and more subtle mechanisms (Stowe *et al.* 2000). As described by the cited authors, plants can exhibit "compensatory growth" after herbivore attack, regenerate new tissue, increase photosynthetic capacity, or compensate for the damage by storing nutrients. However, the real secret underlying how plants develop resistance (tolerance) to herbivore attacks occurs in complex changes in gene expression that have not yet been fully understood (Kessler and Baldwin 2002).

This study evaluated the effects of pine processionary moth (*Thaumetopoea pityocampa* and *Thaumetopoea wilkinsoni*) damage and seasonal environmental factors on photosynthetic pigment concentrations and enzymatic antioxidant activities in *Pinus brutia*. Results showed that pine processionary moth-induced biotic stress caused seasonal variations in chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid levels. In particular, the increase in photosynthetic pigment levels during intense insect damage suggests the activation of plant defense mechanisms. In addition, changes in SOD, POD, CAT, and APX enzyme activities reflect the physiological responses of plants to biotic stress. The increase in the activities of these enzymes in February indicates that plant defense is strengthened during this period when biotic stress is more pronounced.

CONCLUSIONS

1. Damage by the pine processionary moth (*Thaumetopoea* spp.) induces oxidative stress and activation of enzymatic defense systems in *Pinus brutia*. These findings highlight the important ecological and economic impacts of biotic damage on forestry and reveal the critical role of understanding the physiological responses of plants in controlling such pests.
2. The study found that photosynthetic pigment concentrations (chlorophylls and carotenoids) were significantly affected by pine processionary moth damage. During the moth's intense feeding period in February, the levels of chlorophyll-a, total chlorophyll, and carotenoids were higher. This suggests that the plants activate a defense mechanism by increasing pigment production to cope with the stress.
3. Overall, the findings demonstrate a clear link between the biotic stress from the pine processionary moth and the seasonal variations in both photosynthetic pigments and antioxidant enzyme activities within the trees.

ACKNOWLEDGMENTS

Ergin Yilmaz, a PhD student in the Sustainable Forestry Program was supported by the Higher Education Council's 100/2000 Scholarship at Kastamonu University, Institute of Science. This research was financially supported by the Kastamonu University Scientific Research Fund under the project number KÜ-BAP01/2020-43. As the authors, we would like to express our gratitude to Assoc. Prof. Dr. Ferhat Kara, from the Department of Silviculture, Faculty of Forestry, Kastamonu University, for his invaluable support in statistical analysis.

REFERENCES CITED

Agarwal, S., and Pandey, V. (2004). "Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*," *Biologia Plantarum* 48(4), 555-560. DOI: 10.1023/B:BIOP.0000047152.07878.e7

Anetsos, P. K. (1981). "Monograph of *Pinus halepensis* (Mill) and *Pinus brutia* Ten," *Annales Forestales* 9(2), 39-77.

Anonim (Dpt) (1995). *Forestry Report* Devlet Planlama Teşkilatı, Ankara. P.183.

Arbez, M. (1974). "Distribution, ecology and variation of *Pinus brutia* in Turkey," *Forest Genetic Resources Information* 3, FAO Rome: 21-2

Arnon, D. I. (1949). "Copper enzymes in isolated chloroplast Polyphenoloxidase in *Beta vulgaris*," *Plant Physiology* 24, 1-10. DOI: 10.1104/pp.24.1.1

Avci, M. (2000). "Investigation on structure of egg-batches, parasitism and egg laying habits of *Thaumetopoea pityocampa* (Den.&Schiff.) (Lepidoptera: *Thaumetopoeidae*) in various regions of Turkey," *Turkey Journals of Entomology* 24, 167-178.

Battisti, A., Stastny, M., Netherer, S., Robinet, C., Schopf, A., Roques, A., and Larsson, S. (2005). "Expansion of geographic range in the pine processionary moth caused by increased winter temperatures," *Ecological Applications* 15(6), 2084-2096. DOI: 10.1890/04-1903

Boguszewska, D., Grudkowska, M., and Zagdańska, B. (2010). "Drought-responsive antioxidant enzymes in potato (*Solanum tuberosum* L.)," *Potato Research* 53, 373-382. DOI: 10.1007/s11540-010-9178-6

Brett, W. J., and Singer, A. C. (1973). "Chlorophyll concentration in leaves of Juniperus virginiana L., measured over a 2-year period," *American Midland Naturalist* 194-200. DOI: 10.2307/2424280

Carus, S. (2004). "Impact of defoliation by the pine processionary moth (*Thaumetopoea pityocampa*) on radial, height and volume growth of Calabrian pine (*Pinus brutia*) trees in Turkey," *Phytoparasitica* 32, 459-469. DOI: 10.1007/BF02980440

Critchfield, W. B., and Little, E. L. (1966). "Geographic distribution of the pines of the world (No. 991)," *US Department of Agriculture*, Forest Service. DOI: 10.5962/bhl.title.66393

Çanakçıoğlu, H., (1993). "Orman Entomolojisi (Özel Bölüm), İstanbul Üniversitesi Yayın No: 3623," *İstanbul Üniversitesi Fakülte Yayınları* No: 382, 385s.

Denis, J. M., and Schiffermüller, I. (1776). *Systematisches Verzeichniß der Schmetterlinge der Wienergegend herausgegeben von einigen Lehrern am K. K. Theresianum*, Wien, S.1–323.Taf.Ia, Ib.

Devkota, B., and Schmidt, G. H. (1990). "Larval development of *Thaumetopoea pityocampa* (Den. & Schiff.) (Lep., *Thaumetopoeidae*) from Greece as influenced by different host plants under laboratory conditions 1," *Journal of Applied Entomology* 109(1-5), 321-330. DOI: 10.1111/j.1439-0418.1990.tb00059.x

DPT. (2001). "Sekizinci Beş Yıllık Kalkınma Planı, Ormancılık Özel İhtisas Komisyonu Raporu, Devlet Planlama Teşkilatı, yayın no:2531," *Özel İhtisas Komisyonu* 547, Ankara.

Durkaya, A., Durkaya, B., and Dal, İ. (2009). "Length Research Paper The effects of the pine processionary moth on the increment of Crimean pine trees in Bartin, Turkey," *African Journal of Biotechnology* 8(10).

Ebel, J., and Mithöfer, A. (1998). "Early events in the elicitation of plant defense," *Planta* 206, 335-348. DOI: 10.1007/s004250050409

Fady, B., Lefèvre, F., Reynaud, M., Vendramin, G.G., Dagher-Kharrat, M.B., Anzidei, M., and Bariteau, M. (2003). "Gene flow among different taxonomic units: evidence from nuclear and cytoplasmic markers in *Cedrus* plantation forests," *Theoretical and Applied Genetics* 107(6), 1132-1138. DOI: 10.1007/s00122-003-1323-z

Gong, Y., Toivonen, P. M., Lau, O. L., and Wiersma, P. A. (2001). "Antioxidant system level in 'Braeburn' apple is related to its browning disorder," *Botanical Bulletin of Academia Sinica* 42, 259-264.

Gulsen, O., Eickhoff, T., Heng-Moss, T., Shearman, R., Baxendale, F., Sarath, G., and Lee, D. (2010). 42, 259-264. "Characterization of peroxidase changes in resistant and susceptible warm-season turfgrasses challenged by *Blissus occiduus*," *Arthropod-Plant Interactions* 4, 45-55. DOI: 10.1007/s11829-010-9086-3

Halitschke, R., Schittko, U., Pohnert, G., Boland, W., and Baldwin, I. T. (2001). "Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. III. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses," *Plant Physiology* 125(2), 711-717. DOI: 10.1104/pp.125.2.711

Hare, J. D. (2011). "Ecological role of volatiles produced by plants in response to damage by herbivorous insects," *Annual Review of Entomology* 56(1), 161-180. DOI: 10.1146/annurev-ento-120709-144753

Hashemi, S. (2019). "Effect of nanoparticles on lipid peroxidation in plants," in: *Advances in Lipid Metabolism*, IntechOpen, London, UK. DOI: 10.5772/intechopen.88202

Howe, G. A., and Jander, G. (2008). "Plant immunity to insect herbivores," *Annual Review of Plant Biology* 59(1), 41-66. DOI: 10.1146/annurev.arplant.59.032607.092825

Jabs, T., Tschöpe, M., Colling, C., Hahlbrock, K., and Scheel, D. (1997). "Elicitor-stimulated ion fluxes and O_2^- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley," *Proceedings of the National Academy of Sciences* 94(9), 4800-4805. DOI: 10.1073/pnas.94.9.4800

Kanat, M., and Türk, M. (2002). "New cage method for struggling against *Thaumetopoea pityocampa* (Schiff), 109-114," in: *Proceedings of Pine Processionary Moth Symposium*, M. Kanat (Ed), 24-25 April 2002, Kahramanmaraş, Turkey, 226 pp.

Kanat, M., Alma, M. H., and Sivrikaya, F. (2005). "Effect of defoliation by *Thaumetopoea pityocampa* (Den. & Schiff.) (Lepidoptera: *Thaumetopoeidae*) on

annual diameter increment of *Pinus brutia* Ten. in Turkey," *Annals of Forest Science* 62(1), 91-94. DOI: 10.1051/forest:2004095

Kara, N., Korol, L., Isik, K. and Schiller G. (1997). "Genetic diversity in *Pinus brutia* Ten.: Altitudinal variation," *Silvae Genetica* 46 (2-3), 155-1 61.

Katyshev, A. I., Konstantinov, Y. M., and Kobzev, V. F. (2006). "Characterization of Mn-and Cu/Zn-containing superoxide dismutase gene transcripts in *Larix gmelinii*," *Molecular Biology* 40, 327-329. DOI: 10.1134/S0026893306020208

Kaya, Z., and Raynal, D. J. (2001). "Biodiversity and conservation of Turkish forests," *Biological Conservation* 97(2), 131-141. DOI: 10.1016/S0006-3207(00)00069-0

Keeling, C. I., and Bohlmann, J. (2006). "Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defense of conifers against insects and pathogens," *New Phytologist* 170(4), 657-675. DOI: 0.1111/j.1469-8137.2006.01716.x

Kerchev, P., Waszczak, C., Lewandowska, A., Willems, P., Shapiguzov, A., Li, Z., and Van Breusegem, F. (2016). "Lack of glycolate oxıdase1, but not glycolate oxıdase2, attenuates the photorespiratory phenotype of catalase2-deficient Arabidopsis," *Plant Physiology* 171(3), 1704-1719. DOI: 10.1104/pp.16.00359

Kessler, A., and Baldwin, I. T. (2002). "Plant responses to insect herbivory: the emerging molecular analysis," *Annual Review of Plant Biology* 53(1), 299-328. DOI: 10.1146/annurev.arplant.53.100301.135207

Lamb, C., and Dixon, R. A. (1997). "The oxidative burst in plant disease resistance," *Annual Review of Plant Biology* 48(1), 251-275. DOI: 10.1146/annurev.arplant.48.1.251

Leblebici, S. E. M. A., Bozca, F. D., Topkara, E. F., and Yanar, O. (2023). "Comparison of physiological responses in some Pinus species attacked by pine processionary moth," *Russian Journal of Plant Physiology* 70(6), 143. DOI: 10.1134/S1021443723601465

Liu, J., Chen, H., Wang, J., Chen, X., Yang, Z., and Liang, J. (2019). "Photosynthetic traits and antioxidative defense responses of *Pinus yunnanensis* after joint attack by bark beetles *Tomicus yunnanensis* and *T. minor*," *Journal of Forestry Research* 30(6), 2031-2038. DOI: 10.1007/s11676-018-0844-x

Maffei, M. E., Mithöfer, A., and Boland, W. (2007). "Insects feeding on plants: rapid signals and responses preceding the induction of phytochemical release," *Phytochemistry* 68(22-24), 2946-2959. DOI: 10.1016/j.phytochem.2007.07.016

Maffei, M., and Bossi, S. (2006). "Electrophysiology and plant responses to biotic stress," in: *Plant Electrophysiology: Theory and Methods*, Springer Berlin Heidelberg, pp. 461-481. DOI: 10.1007/978-3-540-37843-3_20

Masutti, L., and Battisti, A. (1990). "*Thaumetopoea pityocampa* (Den. & Schiff.) in Italy Bionomics and perspectives of integrated control 1 2," *Journal of Applied Entomology* 110(1-5), 229-234. DOI: 10.1111/j.1439-0418.1990.tb00117.x

Michelozzi, M., Tognetti, R., Maggino, F., and Radicati, M. (2008). "Seasonal variations in monoterpene profiles and ecophysiological traits in Mediterranean pine species of group "halepensis"," *iForest-Biogeosciences and Forestry* 1(1), article 65. DOI: 10.3832/ifor0206-0010065

Milios, E., Kitikidou, K., and Radoglou, K. (2019). "New silvicultural treatments for conifer peri-urban forests having broadleaves in the understory-the first application in the peri-urban of Xanthi in northeastern Greece," *South-east European forestry* Seefor 10(2), 107-116. DOI: 10.15177/seefor.19-16

Mittler, R. (2002). "Oxidative stress, antioxidants and stress tolerance," *Trends in Plant Science* 7(9), 405-410. DOI: 1016/S1360-1385(02)02312-9

Mushtaq, Z., Faizan, S., and Gulzar, B. (2020). "Salt stress, its impacts on plants and the strategies plants are employing against it: A review," *Journal of Applied Biology and Biotechnology* 8(3), 81-91. DOI: 10.7324/JABB.2020.80315

Myteberi, I., Lushaj, B., Laçeja, F., Metaliaj, R., Malsia, V., Mine, V., and Petto, A. (2013). "The presence and the activity of egg-parasitoids of pine processionary moth (*Thaumetopoea pityocampa* Den & Schiff) in new forests of Austrian pine (*Pinus nigra* Arn.) in Korça, Kolonja e Pogradec districts, Albania," *Online International Interdisciplinary Research Journal* 3(6), 34-42.

Nahal, I. (1983). "Le Pin *brutia (Pinus brutia* Ten. subsp. *brutia*). 1ère partie," *Foret Mediterraneenne* (2), 165-172.

Nakano, Y., and Asada, K. (1981). "Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts," *Plant and Cell Physiology* 22(5), 867-880. DOI: 10.1093/oxfordjournals.pcp.a076232

Nouri, K., Nikbakht, A., Haghighi, M., Etemadi, N., Rahimmalek, M., and Szumny, A. (2023). "Screening some pine species from North America and dried zones of western Asia for drought stress tolerance in terms of nutrients status, biochemical and physiological characteristics," *Frontiers in Plant Science* 14, article 1281688. DOI: 10.3389/fpls.2023.1281688

Oppenheimer, H. R. (1967). "Mechanisms of Drought Resistance in Conifers of the Mediterranean Zone and the Arid West of the USA," Part 1: *Physiological and Anatomical Investigations*, Final Report on project No. A10−FS 7, Grant No FG−Is−119, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel.

Orozco-Cardenas, M., and Ryan, C. A. (1999). "Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway," *Proceedings of the National Academy of Sciences* 96(11), 6553-6557. DOI: 10.1073/pnas.96.11.6553

Paniagua Voirol, L. R., Valsamakis, G., Lortzing, V., Weinhold, A., Johnston, P. R., Fatouros, N. E., and Hilker, M. (2020). "Plant responses to insect eggs are not induced by egg-associated microbes, but by a secretion attached to the eggs," *Plant, Cell & Environment* 43(8), 1815-1826. DOI: 10.1111/pce.13746

Peiffer, M., and Felton, G. W. (2005). "The host plant as a factor in the synthesis and secretion of salivary glucose oxidase in larval *Helicoverpa zea*. Archives of ınsect biochemistry and physiology," Published in collaboration with the *Entomological Society of America* 58(2), 106-113. DOI: 10.1002/arch.20034

Peng, J., Deng, X., Jia, S., Huang, J., Miao, X., and Huang, Y. (2004). "Role of salicylic acid in tomato defense against cotton bollworm, *Helicoverpa armigera*, Hubner," *Zeitschrift für Naturforschung C* 59(11-12), 856-862. DOI: 10.1515/znc-2004-11-1215

Powell, G., Tosh, C. R., and Hardie, J. (2006). "Host plant selection by aphids: behavioral, evolutionary, and applied perspectives," *Annual Review of Entomology* 51(1), 309-330. DOI: 10.1146/annurev.ento.51.110104.151107

Prattipati, S. D., Botcha, S., and Maradana, T. N. (2021). "Activities of antioxidant systems during germination of *Sterculia urens* Roxb. Seeds," *Vegetos* 34, 882-888. DOI: 10.1007/s42535-021-00252-8

Pukacki, P. M., and Kamińska-Rożek, E. (2005). "Effect of drought stress on chlorophyll a fluorescence and electrical admittance of shoots in Norway spruce seedlings," *Trees* 19, 539-544. DOI: 10.1007/s00468-005-0412-9

Quezel, P. (1979). "La région méditerranéenne française et ses essences forestières, signification écologique dans le contexte circum-Mediterranean," *Forêt Méditerranéenne* 1(1), 7-18.

Quézel, P. (1985). "Les pins du groupe Halepensis: Ecologie vegetation ecophysiologie," in: *Le pin d'alep et le Pin brutia dans la Sylviculture Méditerranéenne*, Paris, France: Options Méditerranéennes Série Etudes 86, 11-66.

Quézel, P. (2000). "Taxonomy and biogeography of mediterranean pines (*Pinus halepensis* and *P. brutia*)," in: *Ecology, Biogeography and Management of Pinus halepensis and P. brutia Forest Ecosystems in the Mediterranean Basin*, G. Ne'Eman and L. Trabaud (eds.), Buckhuys Publishers, Leiden 1-12. DOI: 10.2307/3236684

Ray, S., Gaffor, I., Acevedo, F. E., Helms, A., Chuang, W. P., Tooker, J., Felton, G. W., and Luthe, D. S. (2015). "Maize plants recognize herbivore-associated cues from caterpillar frass," *Journal of Chemical Ecology* 41, 781-792. DOI: 10.1007/s10886-015-0619-1

Rodríguez-Mahillo, A. I., González-Muñoz, M., Vega, J. M., López, J. A., Yart, A., Kerdelhué, C., Camafeita, E., García Ortiz, J. C., Vogel, H., and Toffolo, E. P. (2012). "Setae from the pine processionary moth (*Thaumetopoea pityocampa*) contain several relevant allergens," *Contact Dermatitis* 67(6), 367-374. DOI: 10.1111/j.1600-0536.2012.02107.x

Sauceda, J. U., Rodriguez, H. G., Lozano, R. R., Silva, I. C., Meza, M. G., and Larga, L. (2008). "Seasonal trends of chlorophylls a and b carotenoids in native trees and shrubs of Northeastern Mexico," *Journal of Biological Sciences* 8(2), 258-267. DOI: 10.3923/jbs.2008.258.267

Selik, M. (1958). "Botanical investigation on *Pinus brutia* especially in comparison with *Pinus halepensis*," *Istanbul University Faculty of Forestry Journal* 8a: 161-198.

Skwarek, M., Patykowski, J., and Witczak, A. (2017). "Changes in antioxidant enzyme activities in *Pinus sylvestris* and *Larix decidua* seedlings after *Melolontha melolontha* attack," *Forest Research Papers* 78, 159-164. DOI: 10.1515/frp-2017-0017

Stowe, K. A., Marquis, R. J., Hochwender, C. G., and Simms, E. L. (2000). "The evolutionary ecology of tolerance to consumer damage," *Annual Review of Ecology And Systematics* 31(1), 565-595. DOI: 10.1146/annurev.ecolsys.31.1.565

Tanaka, R., and Tanaka, A. (2011). "Chlorophyll cycle regulates the construction and destruction of the light-harvesting complexes," *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1807(8), 968-976. DOI: 10.1016/j.bbabio.2011.01.002

Torres, M. A. (2010). "ROS in biotic interactions," *Physiologia plantarum* 138(4), 414-429. DOI: 10.1111/j.1399-3054.2009.01326.x

Usha Rani, P., and Jyothsna, Y. (2010). "Biochemical and enzymatic changes in rice plants as a mechanism of defense," *Acta Physiologiae Plantarum* 32, 695-701. DOI: 10.1007/s11738-009-0449-2

Usta, H. F. (1990). "Kızılçam (*Pinus brutia* Ten.) Ağaçlandırmalarında hasılat Araştırmaları. A study on the yield of *Pinus brutia* Ten. Plantations," *Ormancılık Araştırma Enstitüsü Yayınları Teknik Bülten* 219, 138 P.

Vega, J. M., Moneo, I., Armentia, A., López-Rico, R., Curiel, G., Bartolome, B., and Fernandez, A. (1997). "Anaphylaxis to a pine caterpillar," *Allergy* 52(12). DOI: 10.1111/j.1398-9995.1997.tb02532.x

War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., and Sharma, H. C. (2012). "Mechanisms of plant defense against insect herbivores," *Plant Signaling and Behavior* 7(10), 1306-1320. DOI: 10.4161/psb.21663

Witham, F. H., Blaydes, D. F., and Devlin, R. M., (1971). "Experiments in plant physiology," *Van Nostrand Reinhold Company* New York, pp. 55–56.

Xu, Z., Jiang, Y., and Zhou, G. (2015). "Response and adaptation of photosynthesis, respiration, and antioxidant systems to elevated CO_2 with environmental stress in plants," *Frontiers in Plant Science* 6, article 701. DOI: 10.3389/fpls.2015.00701

Yee, Y., Tam, N. F. Y., Wong, Y. S., and Lu, C. Y., (2002). "Growth and physiological responses of two mangrove species (*Bruguira gymnorrhiza* and *Kandelia candel*) to waterlogging," *Environmental and Experimental Botany* 1-13.

Yordanov, I., Velikova, V., and Tsonev, T. (2000). "Plant responses to drought, acclimation, and stress tolerance," *Photosynthetica* 38, 171-186. DOI: 10.1023/A:1007201411474

Zhang, R. R., Wang, Y. H., Li, T., Tan, G. F., Tao, J. P., Su, X. J., and Xiong, A. S. (2021). "Effects of simulated drought stress on carotenoid contents and expression of related genes in carrot taproots," *Protoplasma* 258, 379-390. DOI: 10.1007/s00709-020-01570-5

Article submitted: June 11, 2025; Peer review completed: August 9, 2025; Revised version received and accepted: August 15, 2025; Published: August 27, 2025.
DOI: 10.15376/biores.20.4.9127-9147