**Potential action of defence enzyme in biotic stress: A review**

ABSTRACT

Plants are the ecosystem's primary source of energy and have a direct or indirect influence on human life. Pathogens and insects’ interference into the plants contribute to enoromous losses in yield and productivity. Pathogens that can invade plants capable of recognizing and responding to their attack by activating security systems. Controls plant defence responses to the net pathogen attacks. Defense enzyme include that Catalase (CAT), superoxide dismutase (SOD), glutathione and other that helps protect the defense enzyme such as Beta-1,3 glucanase phenylalanise ammonia – lyase, peroxide, chitinase, chitosanase, and polyphenol oxidase. Beta- 1,3 glucan and chitin are major polyssacharides in many fungal cell walls. As chitinase and Beta-1,3 glucanase are capable of attacking the fungal pathogen on the cell wall, they are suggested as participating in plant resistance against fungal pathogen. Some important examples of defense enzyme are chitinase, Beta 1,3 Glucanases, Superoxide dismutase, Catalase, Phenylalanine ammonia-lyase (PAL), Peroxide, Glutathione-S-transferase (GST), chitanases- breakdown chitin, a major component of fungal cell walls, helping to protect against fungal infections. Beta-1,3- Glucanases – Degrade Beta-glucans found in the cell walls of various pathogen, including fungi, enhancing the plants defence against infections. Peroxides – Involved in the production of reactive oxygen species to strengthen the cell walls, making it harder the pathogens to penetrate and cause damage to the pathogen cells. Polyphenol oxidases (PPOS)- catalyse the oxidation of phenolic compounds to quinones, and can reinforce cell wall by cross-linking with proteins. Lipoxygenases – Involved in the Synthesis of Jasmonic acid, a signalling molecule that activates defense reason in plants. PAL – Involved in the synthesis of Phenolic compound that contributes to plant defence by forming physical and chemical barrier against the pathogen.

**Keywords:** Biotic stress, enzymes, pathogen, cells, signalling

INTRODUCTION

“Plants are subjected to a plethora of environmental cues that cause extreme losses to crop productivity. Due to fluctuating environmental conditions, plants encounter difficulties in attaining full genetic potential for growth and reproduction. One such environmental condition is the recurrent attack on plants by herbivores and microbial pathogens. To surmount such attacks, plants have developed a complex array of defense mechanisms. The defense mechanism can be either preformed, where toxic secondary metabolites are stored; or can be inducible, where defense is activated upon detection of an attack. Plants sense biotic stress conditions, activate the regulatory or transcriptional machinery, and eventually generate an appropriate response. Plant defense against pathogen attack is well understood, but the interplay and impact of different signals to generate defense responses against biotic stress still remain elusive” (Iqbal et al., 2021; Shehu et al., 2021; Kumar et al., 2023). “In nature, plants face threats from a variety of biotic agents such as pathogens and herbivorous insects that can severely harm host plants” (Ebrahim et al., 2011). The primary strategy for managing plant diseases has been the use of pesticides (Prasannath *et al*., 2014). Nevertheless, there is an increasing worry about finding alternative strategies that seek to reduce the negative effects of pesticides on both the environment and human health. One such eco-friendly method of disease control is the induction of systemic resistance against plant pathogens (De Costa and Prasannath 2015). “When plants are subjected to attacks from pathogens and herbivores, these pressures can trigger biochemical and physiological alterations in plants, such as enhancing the strength of the cell wall through lignification, suberization, and callose deposition; by generating phenolic compounds, phytoalexins, and pathogenesis-related (PR) proteins which subsequently deter various pathogen invasions” (Bowles,1990). Among these, generating and accumulating PR proteins in response to an invading pathogen is crucial (Yadav et al., 2025). “Plants bolster their defense mechanisms by stimulating the activity of a wide array of defense enzymes, which are PR proteins, specifically peroxidase, β-1,3-glucanase, chitinase, polyphenol oxidase, and phenylalanine ammonia lyase that can reduce a herbivore's feeding and also the speed of disease progression” (Vengadaramana, 2017, Kumari and Deborah et al., 2001). “Resistance mediated by host plants against pathogens Interactions between plants and pathogens can result in successful infection (a compatible response) or resistance (an incompatible response). In incompatible interactions, viruses, bacteria, or fungi that infect plants will provoke a series of localized responses within and surrounding the infected host cells. These responses are associated with an oxidative burst (Dixon and Lamb, 1997), which may result in cell death” (Schmelzer and Kombrink, 2001)

**PLANT RESPONSES TO STRESS**

The exposure of plants to adverse environmental conditions enhances the generation of reactive oxygen species (ROS) such as singlet oxygen (1O2), superoxide (O2•-), hydrogen peroxide (H2O2), and hydroxyl radical (OH•). The detoxification process of ROS in plants is crucial for safeguarding plant cells and their organelles from the harmful effects of these species. Variations in subcellular localization and biochemical characteristics of antioxidant enzymes, along with the distinct responses in gene expression and the presence of non-enzymatic mechanisms, lead to a versatile and adaptable antioxidant system capable of regulating optimal ROS levels. The systems for detoxifying ROS encompass enzymatic and non-enzymatic antioxidant elements. Ascorbate and glutathione (GSH), which are non-enzymatic antioxidants, are vital for plant defense against oxidative stress, serving a significant function as antioxidant buffers. Other non-enzymatic antioxidants that contribute include flavonoids, phenolic compounds, alkaloids, tocopherol, and carotenoids. Antioxidants consist of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and peroxidoxin enzymes These enzymes are present in practically all subcellular compartments. Usually, an organelle has more than one enzyme able to scavenge a single ROS. The main hydrogen peroxide-detoxification system in plant chloroplasts is the ascorbate glutathione cycle, in which APX is a key enzyme. APX utilizes a specific electron donor to reduce H2O2 to water. The importance of APX and ascorbate-glutathione cycle is not restricted to chloroplasts; it also plays a role in ROS scavenging in cytosol, mitochondria and peroxisomes. The ROS-scavenging enzymes in plants have been widely studied and the results have demonstrated that, in response to environmental stress, APX activity generally increases along with other enzymes activities, such as CAT, SOD, and GSH reductase. Over the past ten years substantial efforts have been made to understand plant antioxidant systems. The increasing number of publications addressing CAT, SOD, GPX and APX enzymes in plants are examples of this tendency, especially APX, which had the number of articles doubled from 158 published in 2000 to 368 in 2010 (ISI Web of Knowledge database). Publications related more specifically to ROS in plants increased 18 times in the same period. The data presented in this study confirm those -reported by which the number of publications addressing antioxidant mechanisms increased after 2000. This highlights the importance of examining these enzymes to gain deeper insights into the biological processes involved in oxidative stress responses in plants. The aim of this review is to explore the key discoveries regarding the APX enzyme at molecular physiological levels across various plant species. Additionally, the modulation of the APX gene in reaction to abiotic stress factors, particularly temperature, high light, drought, salinity, and heavy metals will also be evaluated.

DEFENSE ENZYME IN THE BIOTIC STRESS

Defense enzymes are crucial for plants' defense against biotic stress. Some significant defense enzymes include catalase, superoxide dismutase, glutathione, and others that aid in protecting the defense enzyme, such as beta-1,3 glucanase, phenylalanine ammonia-lyase, peroxidase, chitinase, chitosanase, and polyphenol oxidase. Beta-1,3 glucan and chitin are principal defense polysaccharides found in numerous fungal cell walls. Some notable defense enzymes are catalase, peroxidase, and superoxide dismutase.

SUPEROXIDE DISMUTASE

“Superoxide dismutase or metalloenzyme (SOD) is the most potent intracellular enzymatic antioxidant, which is universally found in all aerobic organisms and in all subcellular compartments susceptible to ROS-induced oxidative stress. It is widely recognized that various environmental pressures commonly result in heightened ROS production, where SOD is crucial in enhancing plant stress resistance and serves as the initial barrier against the harmful impacts of elevated oxidative stress levels. SOD eliminates O2 by facilitating its dismutation, which is the transformation reaction of one molecule into two others: one O2 molecule is converted to H2O2, while the other is oxidized to O2. The enzyme also eliminates and thus diminishes the likelihood of OH formation through the metal-catalyzed Haber-Weiss reaction, which occurs 10,000 times faster than spontaneous dismutation” (Abbas et al. 2020). “SOD is categorized by their metal cofactors into three distinct types: copper/zinc (Cu/Zn-SOD), manganese (Mn-SOD), and iron (Fe-SOD), which are distributed across different cellular compartments. Three Fe-SOD genes (FSD1, FSD2, and FSD3), three Cu/Zn-SOD genes (CSD1, CSD2, and CSD3), and one Mn-SOD gene (MSD1) were documented in the *A. thaliana* genome” (García Limones *et al*., 2002). “The functionality of SOD isozymes can be revealed through negative staining and characterized by their susceptibility to KCN and H2O2. Mn-SOD is impervious to both inhibitors; Cu/Zn-SOD is vulnerable to both inhibitors; Fe-SOD is immune to KCN and responsive to H2O2. The subcellular localization of these isoenzymes also varies” (Xie *et al*., 2017). “Mn-SOD is located in the mitochondria of eukaryotic cells and in peroxisomes; certain Cu/Zn-SOD isoenzymes are present in cytosolic fractions and chloroplasts of higher plant species” (Rojas Beltran *et al.,* 2000). “Fe-SOD isozymes, which are generally not found in plants, are typically linked to the region of chloroplasts where they occur (Kużniak and Skłodowska 2005). Prokaryotic Mn-SOD and Fe-SOD, along with eukaryotic Cu/Zn-SOD enzymes, are dimers, whereas mitochondrial Mn-SOD exists as tetramers. Peroxisomes and glyoxysomes of Citrullus vulgaris have been shown to exhibit Cu/Zn- and Mn-SOD activity; however, there are no documented instances of extracellular SOD enzymes in plants. All variants of SOD are genetically programmed and are directed to the relevant subcellular compartments utilizing amino-terminal signaling sequences. Various forms of SOD have been cloned from multiple plant species” (Zelko et al. 2002). The upregulation of SOD is associated with the regulation of oxidative stress brought on by biotic and abiotic factors and is vital for plant survival under stress conditions. A notable increase in SOD activity during salt stress was recorded in several plant types, including: Mulberry, *C. arietinum*, and *Lycopersicon esculentum*. Eidogan and Oz (2005) detected “three bands of SOD activity (Mn-SOD, Fe-SOD, and Cu/Zn-SOD) in *C. arietinum* when subjected to salt stress. Additionally, a significant rise in the activity of Cu/Zn-SOD and Mn-SOD isoenzymes was noted under salt stress. Pan et al. (2006) examined the influence of salt and drought stress on *Glycyrrhiza uralensis* Fisch and found a marked increase in SOD activity, but the additional Mn-SOD isoenzyme was identified solely under salt stress. It was concluded that heteromeric FSD2 and FSD3 function as ROS scavengers to support early chloroplast development by shielding chloroplast nucleoids from ROS”. (Syman et al. 2024)

CATALASE

“Catalases are tetrameric gem-containing enzymes that can decompose hydrogen peroxide into water and molecular oxygen, rendering them crucial for ROS detoxification. Catalases possess one of the highest activity coefficients known for enzyme characterization: one CAT molecule can decompose two. 6 six million H2O2 molecules in just 1 one minute. It is critical for catalases to aid in the elimination of hydrogen peroxide concentrated in peroxisomes through oxidases that are involved in beta-oxidation of fatty acids, photorespiration, and purine catabolism. Catalase isoenzymes have been thoroughly examined in higher plants, for instance, *H. vulgare*, in cotyledons of *Helianthus annuus”* (Azpilicueta et al. 2007), and “as many as 12 isozymes have been identified in *Brassica*. Maize has 3 isoforms of catalases (CAT1, CAT2, and CAT3), with their genes being expressed and regulated independently across different chromosomes. CAT1 and CAT2 are found in peroxisomes and cytosol, respectively, whereas CAT3 is located in mitochondria. The *E. coli* catalase is encoded by the katE gene, which is over-expressed in *O. sativa,* contributing to the plant's resistance to salt stress. Moreover, catalases respond to specific hydroxides like methylhydroperoxide (MeOOH). A variable response of catalase was detected under heavy metal stress. This activity decreased in *Glycine max,* *Phragmites australis,* *Capsicum annuum*, and *A. thaliana*, but it increased under cadmium stress in *O. sativa, B. juncea, T. aestivum, C. arietinum*, and *V. mungo* roots” (Singh and Khan 2008). Treating rice seedlings with hydrogen peroxide in non-stressful temperature conditions resulted in elevated catalase activity, which subsequently provided protection to the seedlings against cadmium exposure. Oz and Eidogan (2005) demonstrated in their research that a notable increase in CAT activity was recorded in *C. arietinum* leaves when subjected to salt treatment. Likewise, heightened CAT activity was observed in C. arietinum roots after NaCl and Cu2+ stress. Simova Stoilova et al. (2010) reported “an increase in CAT activity in wheat under drought conditions, particularly high in sensitive varieties. During an investigation of CAT (Cu/Zn SOD) genes in maize, focus was directed towards chloroplasts of Brassica campestris L. ssp. pekinensis cv. Tropical Pride, and it was noted that irradiating plants with SOD CAT B. campestris up to 400 × 10‑9 SO2 also enhances the plants' drought resistance. It was additionally reported that boosting the activity of SOD or CAT individually has little impact on the tolerance to 400 ng mL-1 of SO2 in B. campestris modified with the SOD and CAT genes from E. coli. Co-transformed strains that over-expressed both SOD and CAT were found to exhibit high resistance to SO2” (Tseng et al. 2007)

PEROXIDASE

Ascorbate Peroxidase (APX)

“PX is thought to have the most crucial role in ROS neutralization and safeguarding the cells of higher plants, algae, protozoa, and other organisms. The APX family includes at least five distinct isoforms, such as the thylakoid (tAPX) and glyoxysomal membrane forms (gmAPX), alongside the stromal-soluble chloroplast form (sAPX) and the cytosolic form” (cAPX; Foyer and Noctor 1998). “APX exhibits a greater affinity for H2O2 (mM range) compared to CAT and POD (mM range), and it plays a more significant role in regulating ROS-induced responses during stress. Enhanced expression of APX in plants has been shown under various stress conditions. Increased leaf APX activity under Cd stress has been documented in Ceratophyllum demersum, *B. juncea, T. aestivum,* and *V. mungo”* (Singh et al. 2008). Kao and Hsu (2007) stated that “pretreatment of O. sativa seedlings with H2O2 under non-thermal shock circumstances led to an elevation in APX activity and shielded rice seedlings from subsequent Cd stress. Elevated APX activity was also identified in A. doliolum subjected to salt stress” (Srivastava et al. 2008). A marked increase in APX activity was seen under water stress in three varieties of *P. vulgaris* and *P. asperata.* Sharma and Dubey (2005) observed that “plants resistant to mild drought conditions exhibit heightened APX chloroplastic activity compared to control plants, but activity diminishes with higher drought stress levels. It has been proposed that the overproduction of APX boosts POD activity, which enhances the ROS scavenging system of the organism and contributes to resistance against oomycete pathogens” (Sarowar et al. 2005). “Guaiacol peroxidase (GPOX). APX can be differentiated from those derived from plants by guaiacol peroxidase (GPOX) due to variations in sequences and physiological functions. GPOX degrades indole-3-acetic acid (IAA) and plays a significant part in lignin biosynthesis and defense against biotic stresses by utilizing O2 and H2O2. GPOX favors aromatic electron donors like guaiacol and piragallol, which generally oxidize ascorbate at a rate of around 1% relative to guaiacol” (Asada 1999). “GPOX activity shows considerable variation based on the plant species and the stress condition. An increase in the ARCH content is noted in Cd-exposed plants of T. aestivum, A. thaliana, and C. demersum” (Seo and Cho 2005). Radotic et al. (2000) observed an “initial rise in GPOX activity in spruce needles under Cd-stress, indicating that subsequent Cd treatments led to a decline in activity”. “Concurrent increases in GPOX activity have also been reported in both the leaves and root tissues of Vigna radiate and O. sativa under salt stress. Glutathione reductase (GR) is a flavo-protein oxidoreductase, found in both prokaryotes and eukaryotes. It serves as a potential enzyme in the ASH-GSH cycle and plays a vital role in the defense system against ROS, maintaining a reduced GSH status. Primarily localized in chloroplasts, a small quantity of this enzyme is also present in mitochondria and the cytosol (Koji et al. 2009). The regions of GR1 and GR2 expression in rice, wheat, barley, and corn were investigated through northern blotting, revealing increased regulation of HvGR1, HvGR2, and TaGR2 in response to Fe-deficient conditions rather than Fe-sufficient ones. The expression of eukaryotic GR from B. campestris (BcGR) and E. coli GR (EcGR) was examined in E. coli using pET 28a. Over-expression of BcGR in E. coli demonstrated improved growth and survival compared to the control, but significantly better growth was observed in the E. coli strain transformed with inducible EcGR in the presence of paraquat, SA, and Cd” (Yoon et al. 2005).

CONCLUSION

Plants defend themselves against biotic factors by physically reinforcing the cell wall through lignification, suberization, and the production of diverse PR proteins, including defence-related enzymes such as peroxidase, β-1,3-glucanase, chitinase, phenylalanine ammonia lyase, and polyphenol oxidase in reaction to pathogen invasion. These defense enzymes are also stimulated in plants via the application of exogenous substances, indicating that further research is necessary to explore the defence responses elicited by these treatment agents. Therefore, understanding plant defence-related enzymes can certainly aid in the creation of new control strategies The resistance mediated by the host plant is regulated by defense response genes that encode the production of different pathogenesis-related (PR) proteins. This review primarily discusses the biochemical response of the plant defense mechanism related to defence-associated enzymes that have been recognized as PR proteins. The systemic and induced response provides resistance or tolerance to biotic stresses. The defense proteins that are induced in reaction to an attack deliver impressive protection against pathogens via defense mechanisms.

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