JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2023 | Volume 11 | Issue 1

# Alterations in antioxidant enzyme activities in rice plants treated with various abiotic inducers against the bacterial blight agent *Xanthomonas oryzae* pv. *oryzae*

Original paper

**Leila Esfahani [1], Valiollah Babaeizad [*,1], Heshmatollah Rahimian and [1], Ali Dehestani [2,]**

[1.] Sari Agricultural Sciences and Natural Resources University, Sari, Iran
[2.] Genetics and Agricultural Biotechnology Institute of Tabarestan, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

**Abstract:** Rice is the most important staple food in the world. Bacterial leaf blight of rice, caused by *Xanthamonos oryzae* pv. *oryzae* (Xoo), is a highly destructive and widespread disease. Chemical management approach to control this disease appears ineffective. In this experiment, the effects of three treatments of salicylic acid, potassium phosphite, and chitosan on susceptible rice plants inoculated with the bacteria were investigated to assess the induction of resistance and activity of antioxidant enzymes including catalase (CAT), guaiacol peroxidase (GPX) and superoxide dismutase (SOD) during four days. The results showed that the highest and lowest activity of CAT was recorded in the chitosan and salicylic acid treatment, respectively. The maximum amount of catalase activity was 72 hours after inoculation. Comparison of GPX and SOD enzyme activities at different sampling times revealed that these enzymes reached their highest level at 48 and 72 hours after inoculation across all treatments, respectively. However, among different treatments, the highest activity of these enzymes was observed in plants infected with bacteria under potassium phosphite treatment. The findings show that potassium phosphite increases the activity of plant defense enzymes against the pathogen, ultimately reducing the symptoms of the disease.

**Keywords:** chitosan, induced resistance, *Oryza sativa*, potassium phosphite, salicylic acid.

OPEN ACCESS

**Edited by**
Dr. Shideh Mojerloum,
Aarhus University, Denmark
Shahrood university of technology, Iran

**Date**
Received: 5 September 2023
Accepted: 18 January 2024
Published: 1 February 2024

**Correspondence**
Dr. Valiollah Babaeizad
babaeizad@yahoo.com

**Citation**
Esfahani, L., Babaeizad, V., Rahimian, H. and Dehestani A. (2023). Investigation of changes in antioxidant enzyme activities in rice plants treated with various abiotic inducers against the bacterial blight agent *Xanthomonas oryzae* pv. *oryzae* . *J Plant Mol Breed* 11 (1): 41-53. 10.22058/JPMB.2024.2010427.1284 .

## Introduction

Rice (*Oryza sativa* L.) is one of the most important human food sources, which is widely cultivated all over the world (Ainsworth, 2008). Since the introduction of high-yielding semi-dwarf varieties, diseases and insect pests have increasingly caused severe yield losses year after year (Siddiq and Vemireddy, 2021). Bacteria, viruses, and fungi can cause rice to be susceptible to many diseases (Dai et al., 2007). Bacterial blight of rice *Xanthamonos oryzae* pv. *oryzae* (*Xoo*) is one of the most destructive bacterial diseases of rice in some rice-growing areas in the world, especially tropical Asian areas. This disease can cause as high as 50-70% reduction in rice yield in severe epidemics and it is the second most important rice disease in the world, after Rice Blast (Mew et al., 1992). In response to the invasion of microorganisms, plants use different defense mechanisms to deal with the pathogen. The mechanism of enzymatic antioxidant activities includes the regulation of enzymes like Superoxide dismutase, Catalase, Peroxidase, Glutathione reductase, Glutathione S threonate and Guaiacol peroxidase (Hussain et al., 2016).

Catalase is an important antioxidant inhibitory enzyme which plays a role in the removal of $H_2O_2$. Catalase plays the role of a specific peroxidase (POX) that protects cells against the toxic effects of $H_2O_2$. Catalase has a great affinity for $H_2O_2$, which in turn enables it to form $H_2O$ and $O_2$ (Mittler, 2002; Mastouri et al., 2012). Under stress conditions, guaiacol peroxidase (GPX) reduces the level of $H_2O_2$ in cells (Asada, 1999; Basu et al., 2010). The plant has an immunity system that can be induced by abiotic or abiotic inducers (Buensanteai et al., 2009). Previous studies have shown that priming plants with chemical inducers, such as SA (Ganesan and Thomas, 2001; Li and Zhang, 2012; Saba Anwar et al., 2013), potassium phosphite (Huang et al., 2020) and chitosan (Orzali et al., 2014), is capable of inducing resistance. In rice, the application of SA can induce a SA-dependent signaling pathway that leads to disease resistance. The SA-dependent pathway is associated with systemic acquired resistance (SAR) activated by rice plant pathogens (Sticher et al., 1997). One of the important features of induced resistance is the priming phenomenon, in which rice plants show faster and higher defense responses against bacterial contamination compared to untreated rice plants (Conrath et al., 2002). Chitosan is a linear polysaccharide that may be obtained by deacetylation of chitin, long-chain N-Acetyl- Gluconicamine polymers which can easily be extracted from fungal cell walls and crustacean shells (Badawy and Rabea, 2011). This natural compound plays a role in controlling plant diseases. The mode of action includes a direct antimicrobial activity and an indirect induction of resistance that induces several defense responses in plants (Falcón-Rodríguez et al., 2012). Various studies showed that chitosan prevented the growth of a number of pathogens, including *Xanthomonas* sp. (Li et al., 2008), *Pseudomonas syringae* (Mansilla et al., 2013), *Agrobacterium tumefaciens* and *Erwinia carotovora* (Badawy et al., 2014).

Potassium phosphite is used against soil and airborne fungal and bacterial pathogens. This compound moves in a symplastic way in the plant and has the properties of prevention, immunization, and treatment (Thao and Yamakawa, 2009). Phosphites can have a direct or indirect effect on the pathogen (Deliopoulos et al., 2010). The direct effect includes preventing the growth of fungi and reducing or changing the metabolism of pathogens, and the indirect effect includes stimulating the plant's defense system, such as increasing the production of phytoalexins and reactive oxygen species (ROS), inducing Pathogenesis-Related Proteins (PRs), and strengthening the cell wall (Lobato et al., 2008).

There is no effective chemical control of bacterial blight in rice, and cultivars with good resistance to this disease agent have not been offered (Derakhshan et al., 2020). One of the low cost, healthy ways to fight a number of diseases in plants is through induction of resistance genes using different living and non-living factors. In this study, the possibility of inducing resistance to bacterial leaf blight of rice by using non-living stimuli (salicylic acid, chitosan and potassium phosphite) was evaluated.

## Materials and Methods

Building upon the findings of (Derakhshan et al., 2020), who evaluated the resistance of 24 commercial cultivars of Iranian rice against blight bacteria, the local Tarom cultivar was identified as a

sensitive variety. Consequently, this cultivar was selected for the intended research.

In this study, the standard isolate of *Xanthomonas oryzae* pv. *oryzae* (442) was selected, cultured and propagated in Nutrient Agar Sucrose (NAS) at 29 °C (Derakhshan et al., 2020). To perform the pathogenicity test, a fresh culture of bacteria was used and a suspension with different concentration (OD=0.1, 0.3, 0.5) was prepared. Pathogenicity was confirmed by different methods (suspension injection, sterile scissors, and spray) applied to the local Tarom cultivar (Backer, 2002). In this research, salicylic acid (Merck company) with a concentration of 3 mM, chitosan with a concentration of 400 ppm and potassium phosphite with a concentration of 4 g/liter were used (Katoch et al., 2005; Valadi et al., 2013; Heidarzade et al., 2017). The seeds of local Tarom cultivars were obtained from the Seed Breeding Research Department of the Rice Research Institute of Iran and were disinfected with Tiram's Carboxin 2% solution before cultivation.

Seedlings of local Tarom cv. were inoculated with pathogenic bacteria at the 5-6 leaf stage with a concentration of $10^7$ (OD = 0.1) (17). In the case of salicylic acid, chitosan and potassium phosphite treatments, foliar spray was used 48 hours before pathogen inoculation (Sodhi et al., 2003; Mkhoshkdaman and Pedramfar, 2009). Seedlings were kept in the greenhouse after inoculation with non-living elicitors and pathogenic bacteria. Sampling of leaf tissue of treated and control seedlings (inoculated with bacteria) were done at 0, 48, 72 and 96 hours after inoculation (hai). To investigate the mechanism of resistance, the activity of defense-related enzymes such as catalase (CAT), guaiacol peroxidase (GPX), and superoxide dismutase (SOD) was studied in treatments. This study carried out as factorial experiment in a randomized complete design. All data was analyzed by SAS Software version 9.00. Comparison of the means was performed by Tukey test ($P \leqslant 0.01$). To extract the enzyme solutions, 0.5 g of the leaf sample were ground with a mortar in liquid nitrogen and homogenised with icecold 5ml phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The homogenate was centrifuged at 20,000 RPM for 15 min at 4 °C and supernatant used for assays of the activities of CAT and GPX (Reuveni, 1995) .

Catalase enzyme activity was measured based on Aebi's method (Aebi, 1984). The reaction mixture consisted of 1.5 ml of 100 mM phosphate buffer (pH = 7), 0.5 ml of 7.5 mM hydrogen peroxide and 50 µl of enzyme extract. The final volume was adjusted to 3 ml by adding distilled water. The absorbance changes of the reaction solution were measured by a spectrophotometer (Camspec M501, UV-Vis single beam scanning spectrophotometer, UK) at a wavelength of 240 nm for 2 minutes. To determine the activity of GPX (Chance and Maehly, 1955), the reaction mixture (2 ml) included 1 ml of 100 mM phosphate buffer (pH=7), 250 µl of 0.1 mM EDTA, 1 ml of 5 mM guaiacol, one ml of 15 mM peroxide, and 50 µl of the enzyme solution. The reaction was started by adding the enzyme solution and the increase in absorbance was recorded by a spectrophotometer at a wavelength of 470 nm for 1 minute. Enzyme activity was determined based on the amount of tetragaiacol and obtained using the extinction coefficient of 1.33 µmol/cm. The activity of SOD enzyme was performed using the method of Beyer and Fridovich (Beyer Jr and Fridovich, 1987). 1000 µl of 50 mM phosphate buffer containing 1.5 mM EDTA, 10 mM methionine, and 75 µM nitroblue tetrazolium chloride were mixed with 100 µL of 1 µM riboflavin and 100 µl of enzyme extract. Then the lamp was turned off and the absorbance changes of the reaction mixture were read by a spectrophotometer at a wavelength of 560 nm.

## Results

### *Morphological and pathological features*

In this experiment, the local Tarom cultivar was used as a very susceptible variety against *Xoo* bacterial pathogen (Derakhshan et al., 2020). The pathogenicity of the desired bacterium was proven with a concentration of $10^7$ cfu/ml at the 5-6 leaf stage (Backer, 2002) (Figure 1). Two standard methods of sterile scissors and fogging method with OD = 0.1 equivalent to a suspension with a concentration of $10^7$ cfu/ml were chosen to conduct the experiment. Morphological and pathological characteristics based on Koch's principles were consistent with the results of other researchers (Goto, 1964; Schaad et al., 1996; Backer, 2002). This bacterium causes scorched spots on rice leaves and yellow spots and necrosis appear after 2 weeks.

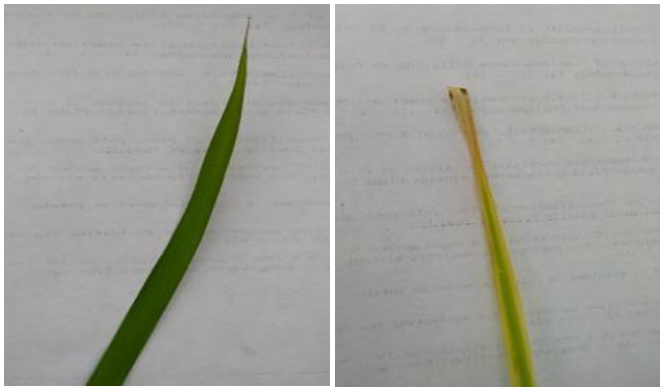

**Figure 1.** Pathogenicity test (Right figure: mock plant; Left: rice plant inoculated with *Xanthomonas oryzae* pv. *oryzae*).

Then the yellow spots spread towards the base of the leaf, and finally more parts of the leaf surface showed yellow symptoms. This bacterium causes scorched spots on rice leaves and within two weeks, yellow spots and necrosis appear. Then, the yellow spots progress in the leaf and spread towards the base, and finally, a larger part of the leaf surface showed yellow symptoms. While in the leaves of the control plants (treated with distilled water), no signs of water-soaked and yellowness were observed.

### *Disease progression*

The results of the analysis of variance of the data showed that there were a significant difference between the treatments. The average comparison between the treatments showed the highest disease progress in the control treatment inoculated with bacteria and the lowest for potassium phosphite treatment (Figure 5). The results of analysis of variance of the data showed that the effect of non-living stimuli treatment (salicylic acid, chitosan, and potassium phosphite), sampling times and the interaction effect of the treatment with time on the activity of CAT, GPX and SOD enzymes in the leaves of local Tarom rice inoculated with *Xoo* at a probability level of one percentage was significant (Table 1). The amount of enzyme activity compared to the control was significant in different treatments and at different times, and it showed that these three treatments increased the activity of defense enzymes (Table 2).

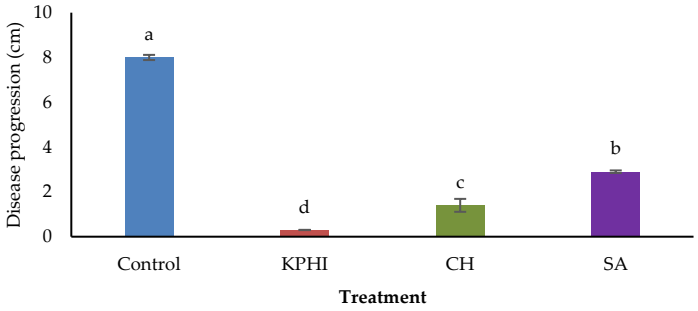


**Figure 5.** Disease progression in the leaves of rice plants treated with potassium phosphite, chitosan and salicylic acid, 11 days after inoculation with Xanthomonas oryzae pv. oryzae. (Control: rice plant inoculated with bacteria, KPHI: rice plant treated with potassium phosphite, CH: rice plant treated with chitosan, SA: rice plant treated with salicylic acid).

**Table 1.** Analysis of variance for activity of defense enzymes of rice plants treated with potassium phosphite, chitosan and salicylic acid against *Xanthamonos oryzae* pv. *oryzae.*

| Source of variations | df | MS | | |
|---|---|---|---|---|
| | | CAT | GPX | SOD |
| Treatment | 3 | 11.44** | 37.35** | 54.46** |
| time | 3 | 0.508** | 22.26** | 36.96** |
| Treatment*time | 9 | 2.43** | 1.80** | 4.26** |
| Error | 32 | 0.005 | 0.19 | 0.009 |
| CV | | 3.93 | 7.64 | 1.77 |

* * Significant at P≤0.01.

**Table 2.** Mean comparison between inducers for CAT, GPX and SOD enzymes CAT, GPX and SOD enzymes.

| Enzymes | Treatments | | | |
|---|---|---|---|---|
| | Potassium phosphite | Chitosan | Salicylic acid | Control (rice plant inoculated with bacteria) |
| CAT | 2.22$^{b}$ | 2.62$^{a}$ | 2.13$^{b}$ | 0.42$^{c}$ |
| GPX | 7.48$^{a}$ | 6.74$^{b}$ | 5.47$^{c}$ | 3.45$^{d}$ |
| SOD | 7.69$^{a}$ | 6.48$^{b}$ | 4.82$^{c}$ | 2.78$^{d}$ |

Comparison of the means was performed by Tukey test (P ≤ 0.01).
Means followed by different letters in each row show significant difference (P≤0.01).

### *CAT activity*

The results of the interaction effect of comparing the average data show that the highest activity of this enzyme was observed in the chitosan treatment (2.62 U mg-1 protein) and the lowest level was observed in the control treatment inoculated with bacteria (0.42 U mg-1 protein), and they were significantly different from each other (Table 1). The highest level of enzyme activity was observed at 72 hours after inoculation (2.08 U mg-1 protein) and the lowest at 0 hours (1.60 U mg-1 protein) (Table 2). Also, the highest effect in increasing the amount of CAT enzyme was observed in the chitosan treatment, which was recorded 48 hours after inoculation (3.48 U mg-1 protein), which was increased 6 times compared to the control treatment infected with bacteria (0.43 U mg-1 protein), and the lowest increase was related to the salicylic acid treatment (1.23 U mg-1 protein) (Figure 2). Salicylic acid treatment recorded the highest amount of CAT enzyme activity at 0 (2.69 U mg-1 protein) and 96 (2.93 U mg-1 protein) hours. Potassium phosphite treatment recorded the maximum activity of CAT enzyme at 72 hours (3.82 U mg-1 protein) and reached the highest value only at this time. In measuring the amount of CAT enzyme among the four sampling times, the maximum average activity of this enzyme was recorded at 72 hours (2.08 U mg-1 protein) after inoculation, after which the enzyme activity decreased at 48 (1.94 U mg-1 protein), 96 (1.78 U mg-1 protein), and 0 (1.60 U mg-1 protein) hours, respectively. Sampling times were significantly different at the one percent probability level (Figure 2).

### *GPX activity*

According to the obtained results of the interaction effect of comparing the average data (Figure 3), the activity level of this enzyme in 48 hours (7.50 U mg-1 protein) after inoculation is at the highest level in all treatments, which has a significant difference with other sampling times at the level of 1% probability (Table 1). The results of the activity of GPX showed that the most effective treatment in increasing the average activity of this enzyme was the potassium phosphite treatment (7.48 U mg-1 protein), which reached its highest level at 48 hours, which was almost three times the control treatment infected with bacteria, which had a significant difference with other treatments at the probability level of 1% (Table 2).

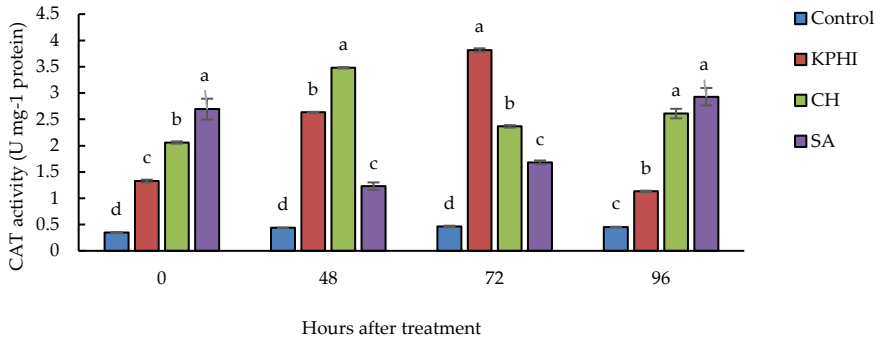


**Figure 2.** Change in catalase activity (CAT) in leaves of rice plants treated with potassium phosphite, chitosan and salicylic acid at hours 0, 48, 72 and 96 after inoculation with *Xanthomonas oryzae* pv. *oryzae*. (Control: rice plant inoculated with bacteria, KPHI: rice plant treated with potassium phosphite, CH: rice plant treated with chitosan, SA: rice plant treated with salicylic acid).

Chitosan (6.74 U mg-1 protein) and salicylic acid (5.47 U mg-1 protein) treatments were ranked second and third, respectively, which had significant differences from each other (Figure 3). The chitosan treatment peaked at 48 hours (9.07 U mg-$^{1}$ protein), which was almost 2.5 times the control treatment infected with bacteria. Salicylic acid treatment recorded the highest activity of GPX enzyme at 48 hours (7.14 U mg-$^{1}$ protein), which was almost twice the control treatment infected with bacteria. The results obtained from the average activity of GPX enzyme showed that among the four sampling times, the activity of the enzyme reached its maximum at 48 hours (7.50 U mg-$^{1}$ protein) and then the enzyme activity decreased at 72 (6.17 U mg-$^{1}$ protein), 96 (5.08 U mg-$^{1}$ protein) and 0 (4.38 U mg-$^{1}$.protein) hours, respectively. Statistically significant difference at the level of 1% was observed between different times (Figure 3).

The results of the interaction effect of comparing the average data showed that the highest activity level of this enzyme (7.69 U mg-$^{1}$ protein) was observed in the potassium phosphite treatment and the lowest level was observed in the control treatment inoculated with bacteria (2.78 U mg-$^{1}$ protein) and they had significant differences with each other (Table 1). The highest activity of this enzyme was observed at 72 hours after inoculation (7.89 U mg-$^{1}$ protein) and the lowest at 0 hours (3.71 U mg-$^{1}$ protein) (Table 3).

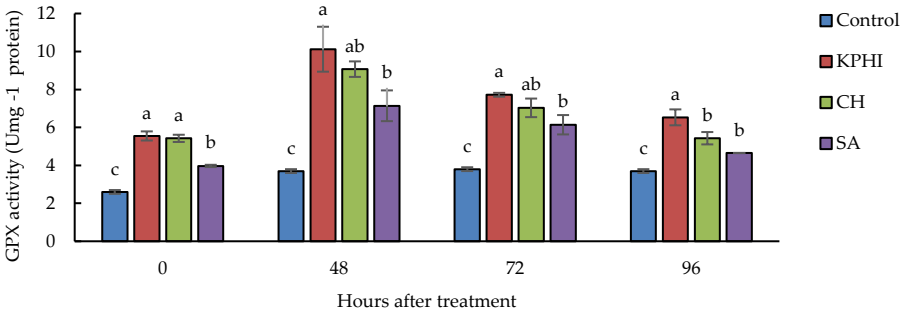


**Figure 3.** Changes in the activity of guaiacol peroxidase (GPX) in the leaves of rice plants treated with potassium phosphite, chitosan and salicylic acid at hours 0, 48, 72 and 96 after inoculation with *Xanthomonas oryzae* pv. *oryzae*. (Control: rice plant inoculated with bacteria, KPHI: rice plant treated with potassium phosphite, CH: rice plant treated with chitosan, SA: rice plant treated with salicylic acid).

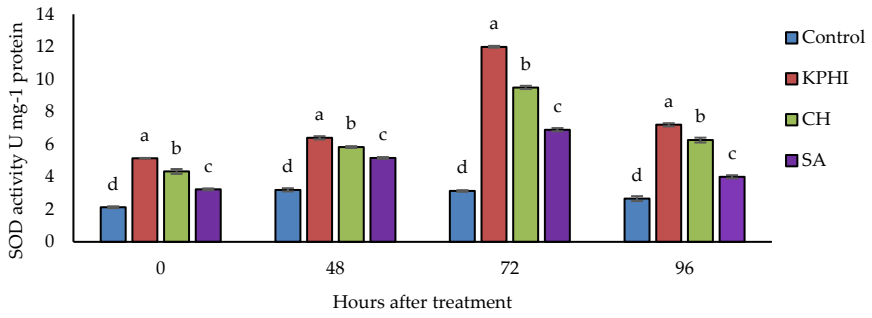


**Figure 4**. Changes in the activity superoxide dismutase (SOD) in the leaves of rice plants treated with potassium phosphite, chitosan and salicylic acid at hours 0, 48, 72 and 96 after inoculation with *Xanthomonas oryzae* pv. *oryzae*. (Control: rice plant inoculated with bacteria, KPHI: rice plant treated with potassium phosphite, CH: rice plant treated with chitosan, SA: rice plant treated with salicylic acid).

**Table 3.** Mean comparison between times after treatment for CAT, GPX and SOD enzymes.

| Enzymes | Hours after treatment | | | |
|---|---|---|---|---|
| | 0 | 48 | 72 | 96 |
| CAT | 1.60$^{d}$ | 1.94$^{b}$ | 2.08$^{a}$ | 1.78$^{c}$ |
| GPX | 4.38$^{d}$ | 7.50$^{a}$ | 6.17$^{b}$ | 5.08$^{c}$ |
| SOD | 3.71$^{c}$ | 5.15$^{b}$ | 7.89$^{a}$ | 5.03$^{b}$ |

Comparison of the means was performed by Tukey test ($P \leqslant 0.01$).
Means followed by different letters in each row show significant difference ($P \leqslant 0.01$).

### *SOD activity*

Also, the highest effect in increasing the SOD enzyme activity was recorded in the potassium phosphite treatment at 72 hours after inoculation (12 U mg-$^{1}$ protein), which was 4 times higher than the control treatment infected with bacteria, and the lowest increase was related to the salicylic acid treatment (6.9 U mg-$^{1}$ protein) (Figure 4). Comparing the average data among the four sampling times, the highest average activity of this enzyme was recorded at 72 hours after inoculation, after which the enzyme activity decreased at 48, 96 and 0 hours, respectively. Statistically significant difference at the level of 1% was observed between different times (Figure 4).

## Discussion

All plants, both resistant and susceptible, respond to pathogen attack by inducing an appropriate signaling system, which leads to the accumulation of different gene products. The response to pathogen attack is effective at different levels: initially, pathogen recognition leads to a rapid local cell death in the plant, also known as the hypersensitive response (HR), which causes necrosis at the site of infection (local response). Then, even in uninfected plant parts, a wide range of systemic expression and long-term resistance to further infection of the pathogen is established. This leads to the production of reactive oxygen species (ROS), the activation of defense-related genes, as well as the increased expression of genes related to the production of molecules, such as phytoalexins, terpenes, pathogenicity-related proteins (PR) and many enzymes involved in defense mechanisms (phenyl Alanine ammonia lyase (PAL), polyphenol oxidases (PPOs) and peroxidases such as guaiacol peroxidase (G-POD) and ascorbate peroxidase (APX) (Heil and Bostock, 2002; Iriti and Faoro, 2009; Pieterse et al., 2009) .

In this experiment, for the CAT enzyme activity, the best treatment in increasing the amount of this enzyme was the chitosan treatment, which recorded the highest amount in 48 hours after inoculation. In plants infected with bacteria and treated with chitosan, the amount of enzyme increased by 87.41% compared to the control plants inoculated with *Xoo* (Figure 2). In the investigation of GPX enzyme activity of plants infected with *Xoo* in chitosan treated plants, it has increased by 59.2% compared to the control plants inoculated with *Xoo* (Figure 3).

Chitosan, with its low molecular weight, acts as a strong biostimulant, capable to inducing plant defense responses and activating various pathways that increase crop resistance to diseases. The most response of the plant to chitosan treatment is the formation of chemical and mechanical barriers and the synthesis of new molecules and enzymes involved in the defense response (Iriti and Faoro, 2009; Siddaiah et al., 2018). In some cases, chitosan induces a hypersensitive reaction, mainly around the site of contamination, leading to programmed cell death. This hypersensitive reaction can be followed by the systemic response of the plant's defense mechanisms. It mainly includes the synthesis and accumulation of secondary metabolites with an active role in defense: phenolic compounds such as lignin, callose, phytoalexins, PR proteins (proteins related to pathogenicity) and modulating the activity of key enzymes of metabolic pathways involved in the defense response, such as PAL, peroxidases and chitinase (Li and Zhu, 2013; Orzali et al., 2014). The results of investigating the effect of chitosan and salicylic acid on the response of rice plants against *Fusarium fujikuroi*, the cause of root and crown rot disease, showed that the two compounds of salicylic acid and chitosan can play an effective role in inducing resistance and reducing the severity of the disease caused by this pathogen (Ebrahimi et al., 2020). The use of chitosan on rice seedlings causes the production of $H_2O_2$, the accumulation of glucanases and chitinase, phenylalanine aminolyase, and also increases the expression of genes involved in pathogenesis (Amborabé et al., 2008; Khatami et al., 2018). The results obtained from this experiment showed that chitosan, in addition to increasing the enzymes mentioned above research, also increases the enzyme CAT and GPX in sensitive rice cultivar (local Tarom) against bacterial leaf blight. These results were consistent with other studies mentioned above (Amborabé et al., 2008; Li and Zhu, 2013; Orzali et al., 2014; Ebrahimi et al., 2020).

In this study, the potassium phosphite treatment was the best treatment in increasing the activity of the GPX enzyme, the maximum of which was observed at 48 hours, which caused a 43.63% increase of this enzyme compared to the control treatment (Figure 3). One of the most effective intracellular antioxidants is SOD enzyme. This enzyme can keep many plants safe from the attack of oxygen free radicals and make the plant stable against many environmental stresses. In a complex reaction, superoxide dismutase combines two superoxide molecules with the help of two hydrogen molecules and turns them into two hydrogen peroxide molecules which in the next step is removed by other antioxidants (Lin et al., 2011). In this research, potassium phosphite increases SOD enzyme activity 72 hours after infection, which caused a 73.98% increase of this enzyme compared to the control treatment. This enzyme can limit the pathogenic mechanism of bacteria by creating unfavorable conditions. If phosphite (PHI) is applied at appropriate concentrations, it can stimulate defense responses in plants. Some chemicals such as PHI at lower concentrations have been widely used in the management of *Phytophthora* spp. (Guest and Grant, 1991) . The protective pattern of PHI application mainly involves the direct inhibition of pathogen growth, the accumulation of stress-related metabolism, and the expression of defense genes (Daniel and Guest, 2005; Habibi Daronkolaei et al., 2023). The protection of PHI application on pathogens is dose-dependent. A low concentration of PHI (10 µg/ml) causes the synthesis of defense genes, phytoalexins and phenolic compounds. While under high concentrations (100 µg/ml), PHI can directly inhibit the growth of pathogens (Daniel and Guest, 2005; Dalio et al., 2014). PHI induces resistance to pathogen attack through priming. Priming is a process in which plants gain more resistance to pathogens by the action of some plant hormones and other chemical compounds (Conrath et al., 2002). ROS production increases and callose and pathogenicity-related genes accumulate in priming

responses. Under low concentrations of PHI and SA, pathogenicity-associated protein 1 accumulated and increased resistance to future pathogen attack (Van Loon et al., 1998; Conrath et al., 2002). Another report (Machinandiarena et al., 2012) showed that the application of Phi reduces the accumulation of $H_2O_2$ in fresh plant tissues.

Potassium phosphite ($KH_2PO_3$ or KPhi) has a high potential to control *Phytophthora infestans* due to its direct and indirect effects, which causes indirect effects by stimulating plant defense mechanisms, inducing hypersensitive reactions (HRs) and accumulation of phenylpropanoid biosynthetic enzymes to ultimately prevent the development of late blight (Eshraghi et al., 2011). The results of investigating the effect of five levels of potassium phosphite treatment at five different times in cucumber plants inoculated with *Fusarium oxysporum* f. sp. *cucumerinum-radicis* showed that compared to the control plants, the activity of all defense enzymes and metabolites investigated including GPX, CAT, SOD and also the amount of metabolites such as malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) increased significantly as a result of different potassium phosphite and *Fusarium oxysporum* f. sp. *cucumerinum-radicis* (Heidarzade et al., 2017). The results obtained from our research were consistent with other studies mentioned above.

In this experiment, the activity of CAT enzyme in plants infected with *Xoo* under salicylic acid treatment increased by 87.15% at zero hour and by 84.53% at 96 hours after teatment compared to the control (inoculated with bacteria) (Figure 2). Also, the activity of GPX enzyme in plants infected with bacteria and treated with salicylic acid increased by 48.17% compared to control (inoculated with bacteria) at 48 hours (Figure 3). Foliar spraying with SA causes the production of proteins with different functions, including signal transduction, antioxidant and defense activity. Glucosyltransferase enzyme production was maximally induced by 1 mM SA, with a 7-fold increase in enzyme activity at 6 h in both roots and shoots after SA treatment (Silverman et al., 1995). However, increased peroxidase activity was recorded after foliar application of 8 mM SA in rice to induce resistance to rice blast (Daw et al., 2008). The results obtained from our experiment were consistent with the studies mentioned above.

## Conclusion

In this research, the applied treatments have led to an increase in the activity of defense enzymes, effectively impeding the advancement of *Xanthomonas oryzae* pv. *oryzae* bacteria. The results of this research indicate that pretreating rice plants with chitosan, potassium phosphite and salicylic acid enhances resistance against the bacterium *Xanthomonas oryzae* pv. *oryzae*, the causative agent of bacterial leaf blight of rice, through increasing the activity of defense enzymes. The obtained results showed that chitosan was the most effective in increasing CAT enzyme activity, while potassium phosphite demonstrated superior efficacy in promoting GPX and SOD enzyme activities. The highest activity of antioxidant enzymes CAT and GPX occurred 48 hours after inoculation, while for the SOD enzyme, the peak activity was observed 72 hours after inoculation of rice plants with *Xanthomonas oryzae* pv. *oryzae* bacteria. Increasing the amount of antioxidant enzymes is one of the mechanisms of plant resistance against pathogens. In the treated plants, the enzyme activity increased compared to the infected control plants. This suggests that the applied treatments stimulate greater enzyme activity, consequently enhancing the plant's resistance to the pathogen.

## Supplementary Materials

No supplementary material is available for this article.

## Author contributions

Conceptualization, L.E. and V.B.; methodology, L.E.; software, L.E.; validation, L.E., V.B. and A.D.; formal analysis, L.E. and V.B.; investigation, L.E.; resources, V.B.; data curation, , L.E. and V.B.; writing—original draft preparation, , L.E. and V.B.; writing—review and editing, , L.E. and V.B.; visualization, , L.E. and V.B.; supervision, L.E., V.B., H.R. and A.D.; project administration, L.E., V.B., H.R. and A.D.; funding acquisition, L.E., V.B. and A.D.

## Funding

This research received no external funding.

## Acknowledgments

## Conflict of interest statement

The authors declare no conflict of interest.

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JPMB
Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2023 | Volume 11 | Issue 1

مقاله پژوهشی

# تغییرات فعالیت آنزیم‌های آنتی‌اکسیدانی در گیاهان برنج تیمارشده با القاکننده‌های غیرزنده مختلف در برابر عامل سوختگی باکتریایی *Xanthomonas oryzae* pv. *oryzae*

**لیلا اصفهانی [۱]، ولی‌اله بابایی‌زاد *[۱]، حشمت‌اله رحیمیان [۱] و علی دهستانی [۲]**

[۱]. گروه گیاه‌پزشکی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

[2]. پژوهشکده ژنتیک و زیست‌فناوری کشاورزی طبرستان، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران

**چکیده:** برنج (*Oryzae sativa* L.) مهم‌ترین غذای اصلی در جهان است. سوختگی برگ باکتریایی (BLB) برنج ناشی از *X. oryzae* pv. *oryzae* یک بیماری بسیار مخرب و گسترده است. مدیریت شیمیایی برای کنترل این بیماری ناکارآمد به نظر می‌رسد. در این آزمایش تاثیر سه تیمار سالیسیلیک‌اسید، فسفیت‌پتاسیم و کیتوزان در گیاه برنج حساس تلقیح شده با باکتری *Xanthamonos oryzae* pv. *Oryzae* بر القای مقاومت و فعالیت آنزیم‌هایی مانند کاتالاز (CAT)، گایاکول‌پراکسیداز (GPX) و سوپراکسیددیسموتاز (SOD) در طی چهار روز بررسی شد. نتایج نشان داد که بیشترین و کمترین فعالیت CAT به ترتیب در تیمار کیتوزان و اسیدسالیسیلیک ثبت شد. حداکثر میزان فعالیت کاتالاز ۷۲ ساعت پس از تلقیح بود. مقایسه فعالیت آنزیم‌های GPX و SOD در زمان‌های مختلف نمونه‌برداری نشان داد که این آنزیم‌ها به ترتیب در ۴۸ ساعت و ۷۲ ساعت ساعت پس از تلقیح در تمامی تیمارها در بالاترین سطح قرار دارند. اما در بین تیمارهای مختلف، بیشترین فعالیت این آنزیم‌ها در گیاهان آلوده به باکتری تحت تیمار فسفیت‌پتاسیم مشاهده شد. یافته‌ها نشان می‌دهد که از بین سه تیمار سالیسیلیک‌اسید، فسفیت‌پتاسیم و کیتوزان، تیمار فسفیت‌پتاسیم باعث افزایش فعالیت آنزیم‌های دفاعی گیاه در برابر عامل بیماری‌زا می‌شود و سپس منجر به محدود کردن عامل بیماری‌زا و کاهش علائم بیماری می‌شود.

**کلمات کلیدی:** *Oryzae sativa*، اسیدسالیسیلیک، فسفیت‌پتاسیم، کیتوزان، مقاومت القایی.

**ویراستار علمی**
دکتر شیده موجرلو،
دانشگاه آرهوس، دانمارک
دانشگاه صنعتی شاهرود، ایران

**تاریخ**
دریافت: ۱۴ شهریور ۱۴۰۲
پذیرش: ۲۸ دی ۱۴۰۲
چاپ: ۱۱ بهمن ۱۴۰۲

**نویسنده مسئول**
دکتر ولی‌اله بابایی‌زاد

babaeizad@yahoo.com

**ارجاع به این مقاله**

Esfahani, L., Babaeizad, V., Rahimian, H. and Dehestani A. (2023). Investigation of changes in antioxidant enzyme activities in rice plants treated with various abiotic inducers against the bacterial blight agent *Xanthomonas oryzae* pv. *oryzae* . *J Plant Mol Breed* 11 (1): 41-53. 10.22058/JPMB.2024.2010427.1284 .