Plant Sci. Arc.
Plant Science Archives
An International Journal of Plant Biology

Original Research Article
ISSN 2518-6965
www.plantarc.com

Effect of resistance inducing chemicals and seaweed extracts on defence enzymes in *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) challenged paddy plants

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Citation: C. Kannan (2017). Effect of resistance inducing chemicals and seaweed extracts on defence enzymes in *Xanthomonas oryzae pv. oryzae (Xoo)* challenged paddy plants.
Plant Science Archives. **01-03. DOI: https://doi.org/10.51470/PSA.2017.2.1.01**

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Received 15 December 2016 | Revised 11 January 2017 | Accepted 05 February 2017 | Available Online March 06 2017

ABSTRACT

Several chemicals, including salicylic acid, acibenzolar-S-methyl, acetylsalicylic acid, nicotinic acid, jasmonic acid, and oxalic acid, have been shown to induce resistance in various crops. Marine algae, with their vast array of complex natural products, represent a promising source of novel bioactive compounds that can enhance plant resilience by providing protection against stress induced by pathogens. This study aimed to evaluate the effects of various resistance-inducing chemicals and seaweed extracts on the activation of defense enzymes in Xanthomonas oryzae pv. oryzae (Xoo)-challenged paddy plants. The findings may contribute to the development of eco-friendly strategies for managing bacterial diseases in rice cultivation.

Keywords: *Xanthomonas oryzae, seaweed, enzymes, paddy plants*

Introduction

Bacterial leaf blight (BLB), a devastating disease in rice, has traditionally been managed using chemical treatments like copper oxychloride and streptomycin sulphate. However, excessive reliance on these chemicals poses significant environmental risks, affects consumer health, and damages beneficial predators and parasitoids essential for the ecosystem [1]. As a result, there is growing interest in eco-friendly alternatives for pathogen control that promote both crop yield and sustainable crop health.

Marine algae, including green, brown, and red seaweeds, have emerged as a promising source of bioactive compounds with antimicrobial potential against pathogenic microbes of medical, agricultural, and environmental significance [2]. Several substances extracted from seaweeds have demonstrated antibacterial and antifungal activities, as well as the ability to inhibit pathogen growth [3]. Beyond seaweed extracts, the induction of resistance through chemicals offers an alternative approach to plant protection. Resistance-inducing chemicals act as inducers of phytoalexins or elicit resistance responses in various plant species [4-6].

Several compounds, including salicylic acid [7], acibenzolar-S-methyl [8], acetylsalicylic acid [9], nicotinic acid [10], jasmonic acid [3] and oxalic acid [7], have been shown to induce resistance in crops. These chemicals can enhance plant defense mechanisms by triggering the production of phytoalexins and other resistance-related enzymes, offering a sustainable approach to disease management. This study aims to evaluate the effectiveness of various resistance-inducing chemicals and seaweed extracts on defense enzyme activation in **Xanthomonas oryzae** pv. **oryzae** (Xoo)-challenged rice plants.

Materials and Methods

Pot Culture Studies

The effectiveness of various seaweed extracts and certain resistance-inducing chemicals was evaluated for their ability to suppress the in vitro growth of *Xanthomonas oryzae* (Xoo) and assess their impact on the incidence of bacterial blight diseases under greenhouse conditions. The susceptible rice variety ADT 38 was grown in pots for this study.

To prepare the inoculum, **Xoo** was sub-cultured, and the bacterial load was adjusted to 1 × 10^8 CFU/ml by adding sterile distilled water. Inoculation was performed on thirty-five-day-old plant leaves using the scissors-dip method, following the standard protocol outlined by [4]. The plants were maintained in a polyhouse with frequent watering to ensure adequate moisture and relative humidity, facilitating successful infection by the pathogen.

Resistance-inducing chemicals, including salicylic acid and abscisic acid, were applied at concentrations of 50 ppm and 75 ppm, respectively. The effective seaweed extract, ***Sargassum wightii***, was applied at a concentration of 10% (v/v). All treatments were sprayed individually at the disease initiation stage and were reapplied once every fifteen days. Each treatment was replicated three times, with a control group maintained for comparison. Additionally, streptomycin sulfate was used at a concentration of 100 ppm as a standard antibiotic treatment.

T_1- Salicylic acid 75 ppm
T_2- Abscisic acid 75 ppm
T_3- *Sargassum wightii*@ 10 %
T_4- Salicylic acid 75 ppm + *Sargassum wightii*@ 10 %
T_5- Abscisic acid 75ppm + *Sargassum wightii*@ 10 %
T_6- Streptomycinsulphate 100 ppm.
T_7- Control.

Défense Enzymes

Sample collection

Plant material samples from each treatment were collected at various time intervals (0, 1, 3, 5, and 7 days) after inoculation. To preserve the enzymatic and biochemical constituents, the samples were quickly frozen in liquid nitrogen and stored at -20°C. Subsequent analyses will focus on measuring key enzymatic and biochemical changes, including peroxidase,

polyphenol oxidase, phenylalanine ammonia lyase, β-1,3-glucanase, and total phenols. This approach will allow for a comprehensive assessment of the effects of the treatments over time.

Enzyme extraction

Leaf tissues collected from the plants were immediately weighed, and a 1-gram sample was macerated with 2 ml of sodium phosphate buffer (0.1 M, pH 7.0) at 4°C. The homogenate was then centrifuged at 10,000 rpm for 20 minutes to separate the supernatant. The resulting extracts were utilized for the estimation of key enzymes, including peroxidase (PO), polyphenol oxidase (PPO), and L-phenylalanine ammonia-lyase (PAL).

Spectrophotometric assay

Peroxidase Activity Assay

Peroxidase activity was measured spectrophotometrically following the method described by Hartee (1955). The reaction mixture comprised 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract, and 0.5 ml of 1% hydrogen peroxide (H_2O_2). The mixture was incubated at room temperature (28 ± 1°C). Changes in absorbance at 420 nm were recorded at 30-second intervals over a duration of 3 minutes, with the boiled enzyme preparation serving as a blank control. Enzyme activity was expressed as the change in absorbance of the reaction mixture per minute per gram of fresh weight [8].

Polyphenol Oxidase (PPO) Activity Assay

Polyphenol oxidase (PPO) activity was assessed following the procedure outlined by Mayer et al. (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. The reaction was initiated by adding 200 µl of 0.01 M catechol. PPO activity was expressed as the change in absorbance at 495 nm per minute per gram of fresh weight of tissue.

Phenylalanine Ammonia-Lyase (PAL) Activity Assay

The assay for phenylalanine ammonia-lyase (PAL) activity was conducted according to the method described by Ross and Sederoff (1992). The assay mixture consisted of 100 µl of enzyme extract, 500 µl of 50 mM Tris-HCl buffer (pH 8.8), and 600 µl of 1 mM L-phenylalanine. The reaction was incubated for 60 minutes at room temperature, after which it was arrested by adding 2 N HCl. Subsequently, 1.5 ml of toluene was added, and the mixture was vortexed for 30 seconds and then centrifuged at 1000 rpm for 5 minutes to separate the phases. The toluene phase containing trans-cinnamic acid was measured at 290 nm against a blank of toluene. A standard curve was generated using graded amounts of cinnamic acid in toluene, as previously described. The enzyme activity was expressed as nanomoles of cinnamic acid produced per minute per gram of fresh tissue.

Result and Discussion

Changes in peroxidase activity in *Xoo* challenged paddy plants treated with different formulations under pot culture condition

The application of resistance-inducing chemicals and seaweed extracts at various dosages in paddy plants resulted in significant changes in peroxidase enzyme activity, as shown in Table 1. Notably, the combined application of Salicylic acid at 75 ppm and Sargassum wightii led to a marked increase in peroxidase activity up to the fifth day post-challenge inoculation compared to the control group. The peroxidase activity peaked on the fifth day following pathogen inoculation. In contrast, enzyme activity in the control group decreased by the fifth and seventh days, while treatments with seaweed extracts and resistance-inducing chemicals maintained or enhanced peroxidase activity.

[3] reported that seaweed extracts elevated chitinase and peroxidase activities, subsequently reducing the incidence of powdery and downy mildew in melon leaves. [5] found that the foliar application of salicylic acid increased resistance in wheat plants against powdery mildew pathogens. These findings suggest a strong correlation between the reduction in disease incidence and the enhancement of peroxidase activity in treated plants.

Changes in polyphenol oxidase activity in *Xoo* challenged paddy plants treated with different formulations under pot culture condition

The increased activity of polyphenol oxidase (PPO) was observed in paddy plants challenged with *Xanthomonas oryzae pv. oryzae* (Xoo). The application of Salicylic acid at 75 ppm in conjunction with Sargassum wightii at a concentration of 10% resulted in significantly elevated PPO activity up to the fifth day post-inoculation compared to the control group (Table 2). This was closely followed by the application of Abscisic acid at 75 ppm along with *Sargassum ilicifolium* at 10%, which also showed increased PPO activity in challenged plants. Conversely, plants inoculated solely with the pathogen exhibited relatively low PPO activity, which was notably diminished on the fifth day after inoculation, with further decline observed in the control group over time.

In contrast, the PPO activity increased with age in plants treated with seaweed extracts, highlighting their antagonistic effects and potential in controlling bacterial leaf blight (BLB) disease. [8] reported that increased phenylalanine ammonia-lyase (PAL) activity was observed in chili plants treated with seaweed extracts and challenged with Colletotrichum capsici. Additionally, [3] found that Salicylic acid-treated plants exhibited increased levels of peroxidase, PPO, and ascorbic acid oxidase when inoculated with Corynespora personatum in groundnut.

Changes in phenylalanine ammonia-lyase activity in *Xoo* challenged paddy plants treated with different formulations under pot culture condition

The results indicated that the application of Salicylic acid at 75 ppm in conjunction with *Sargassum wightii* at 10% effectively activated phenylalanine ammonia-lyase (PAL) in the leaves of paddy plants. Inoculation with Xanthomonas oryzae pv. oryzae (Xoo) prompted a rapid and transient accumulation of PAL at the site of infection. Furthermore, PAL activity exhibited a time-dependent increase, peaking on the fifth day post-infection. In contrast, plants inoculated solely with the pathogen displayed a brief increase in PAL activity during the first day, followed by a significant decline thereafter (Table 3).

[6] previously reported enhanced PAL activity in Salicylic acid-pretreated okra plants challenged with Erysiphe cichoracearum. Additionally, treatment with seaweed extracts significantly elevated the activity of several defense-related enzymes, including peroxidase, polyphenol oxidase, chitinase, and β-1,3-glucanase [1-4].

Table 1. Changes in peroxidase activity in Xoo challenged paddy plants treated with different formulations

Treatments	PO activity in plants				
	Time interval (days)				
	0	1	3	5	7
T_1 – Salicylic acid 75 ppm	0.383	0.534	0.686	0.800	0.762
T_2 – Abscisic Acid 75 ppm	0.372	0.509	0.632	0.784	0.729
T_3 – *Sargassum wightii* @ 10 %	0.391	0.554	0.887	0.995	0.972
T_4 – Salicylic acid 75 ppm + *Sargassum wightii*@ 10 %	0.401	0.587	0.961	1.286	1.236
T_5 – Abscisic Acid 75 ppm + *Sargassum wightii*@ 10 %	0.398	0.573	0.935	1.212	1.200
T_6 –Streptomycin (100 ppm)	0.397	0.568	0.911	1.163	1.133
T_7 – Inoculated control	0.148	0.191	0.172	0.147	0.106

Table 2. Changes in polyphenol oxidase activity in Xoo challenged paddy plants treated with different formulations

Treatments	PPO activity in plants				
	Time interval (days)				
	0	1	3	5	7
T_1 – Salicylic acid 75 ppm	0.283	0.527	0.613	0.708	0.653
T_2 – Abscisic Acid 75 ppm	0.262	0.431	0.582	0.632	0.588
T_3 – *Sargassum wightii* @ 10 %	0.326	0.630	0.820	0.913	0.852
T_4 – Salicylic acid 75 ppm + *Sargassum wightii*@ 10 %	0.412	0.825	0.990	1.199	1.168
T_5 – Abscisic Acid 75 ppm + *Sargassum wightii*@ 10 %	0.391	0.800	0.972	1.161	1.123
T_6 –Streptomycin (100 ppm)	0.330	0.635	0.841	0.930	0.902
T_7 – Inoculated control	0.185	0.237	0.242	0.195	0.180

Table 3. Changes in phenylalanine ammonia-lyase activity in Xoo challenged paddy plants treated with different formulations

Treatments	PAL activity in plants				
	Time interval (days)				
	0	1	3	5	7
T_1 – Salicylic acid 75 ppm	0.498	0.600	0.710	0.827	0.800
T_2 – Abscisic Acid 75 ppm	0.461	0.583	0.695	0.800	0.781
T_3 – *Sargassum wightii* @ 10 %	0.597	0.682	0.781	0.957	0.916
T_4 – Salicylic acid 75 ppm + *Sargassum wightii*@ 10 %	0.699	0.789	0.880	1.190	1.095
T_5 – Abscisic Acid 75 ppm + *Sargassum wightii*@ 10 %	0.642	0.710	0.822	1.113	1.023
T_6 –Streptomycin (100 ppm)	0.563	0.670	0.761	0.870	0.852
T_7 – Inoculated control	0.382	0.392	0.360	0.343	0.320

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