International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 05-05-2022, Accepted: 21-05-2022, Published: 06-06-2022
Volume 7, Issue 6, 2022, Page No. 17-19

Effect of *Pseudomonas* exopolysaccharides (an elicitor) on the enhancement of plant growth and biocontrol against *Pyricularia oryzae* of rice var. (ADT-36)

P Manikandan, S Kalaiarasu
Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India

Abstract
A study on the incidence of blast disease in rice using different concentrations of purified exopolysaccharides (100, 200, and 300 ppm) when compared to other concentrations, the use of the same at a concentration of 200 ppm effectively controlled the disease incidence to a greater extent. The results also showed that pseudomonas exopolysaccharides have a dual effect on host plant growth enhancement and Pyricularia oryzae biocontrol, whereas the use of Induced Systemic Resistance inducing chemicals was limited to a reduction in blast disease incidence. Interestingly, pseudomonas exopolysaccharides at 200 ppm were found to be effective in controlling blast disease in upland rice.

Keywords: pseudomonas, exopolysaccharides, and blast disease biocontrol

Introduction
Rice (Oryza sativa L.) is grown in both upland and lowland conditions in India, with rained upland conditions accounting for 13% of the 44 million hectares of rice cultivated area. One of the most common diseases caused by Pyricularia oryzae is blast disease in damaging fungal diseases of upland rice crops, causing up to 90% yield loss. ISR mediated by PGPR appears to be anti- blast pathogen appears to be a promising strategy for reducing the biological and environmental risks posed by synthetic chemical pesticides. Using "exopolysaccharides mediated pseudomonas as a delivery system for the enhancement of crop plant growth and yield under stress conditions such as moisture and temperature," Neyra *et al*. (1995) proposed. Despite the fact that many reports suggest that pseudomonas inoculation has a beneficial effect on rice crops, the role of exopolysaccharides – pseudomonas flocculated culture rich application on systemic resistance induction (ISR) against Pyricularia there has been no research into oryzae in upland rice. The goal of this study was to see if Pseudomonas exopolysaccharides could be used as an elicitor for (ISR) in upland rice crops against Pyricularia oryzae

Materials and Methods
Preparation *Pseudomonas* Strains
Pseudomonas strains Pseu-7, Pseu-18, Pseu-26, and Pseu-37 were isolated from the upland rice rhizosphere. ADT-36 was employed. All pseudomonas isolates were grown separately for 24 hours in a shaking bath at 30 20oC in synthetic malate broth with 0.05 percent yeast extract (W/V) (Day and Dobereiner, 1976) [2]. After centrifuging the medium at 5000 x g for 10 minutes to collect the pellets were washed three times with 0.1 M phosphate buffer before being placed in log phase cells (pH 6.8)before being resuspended in the same buffer to a cell concentration of 1 x 109 CFU/mL measured at 420 nm and used as inoculums.

Pseudomonas Exopolysaccharides
The current study used a minimal salts medium with 8mM fructose and 0.5mM KNO3 as the sole carbon and nitrogen sources (Neyra and van Berkum, 1977) [6]. Each Pseudomonas strain culture (1 x 10-7 CFU/ml) In a 250ml Erlenmeyer flask, 100ml of fructose medium was added and incubated for 5 days at 30°C.2oC while shaking (250rpm) in a rotary shaker. Following incubation, the Exopolysaccharides produced by individual pseudomonas strains were extracted and purified in the manner described by Kyungseok *et al*. (2008) [5] and used at three concentrations: 100, 200, and 300ppm.

Preparation of Induced Systemic Resistance Chemicals
To induce ISR, 0.01 percent salicylic acid, jasmonic acid, and pseudomonas (Himedia, India) were used.

Treatments
The following treatments were used to evaluate Pyricularia oryzae biocontrol ability: The optimization of different concentrations of pseudomonas exopolysaccharides blast disease incidence was tested at concentrations

of 100, 200, and 300 ppm, with ISR inducing chemicals at 0.01 percentage concentration and pseudomonas exopolysaccharides at 200 ppm.

Preparation of Growth Chamber

Two desiccators served as the growth chamber (12 x 10 cm). Weaver's medium was used to fill the lower section, and stainless steel wire mesh was used to support the upper section (mesh size 3 mm). The cotton was covered with the lid, and the chamber was seale prior to sterilisation. The growth chamber was sterilised using autoclaving. After sterilising the growth chamber, fifty germinated rice seeds with coleoptiles (2 cm tall) were incubated for ten days on stainless steel wire mesh. The growth chamber was run on a 14-hour day and 10-hour night cycle, with temperatures ranging from 24oC at night to 32oC around noon. By this point, the rice roots had produced a large number of lateral roots that were well spread in the Weaver's medium kept in the lower part of the growth chamber.

Inoculation of Rice Crop with Pyricularia Oryzae

P oryzae AU – 1 was grown in oat meal agar (OMA) medium and used for challenge inoculation (provided by Annamalai University's Department of Plant Pathology). To obtain a free suspension of conidia, a thick spore suspension was prepared with sterile distilled water from a 10-day-old culture maintained in OMA medium and strained through a double layer muslin cloth. A spore suspension with an optimum spore concentration (50,000 spore'sml-1) was prepared after adjusting the population with a haemocytometer. The spore suspension was then mixed with a few drops of Tween – 80, which increased spore adherence and served as a sticker. Under proper humid conditions, the spore suspension was sprayed. On control plants, sterile distilled water was also sprayed. After one week of challenge inoculation, the incidence of blast disease was recorded using a 0-9 grade scale devised by the International Rice Research Institute (1980) [4]. The statistical analysis was performed in accordance with Gomez and Gomez (1984) [3].

Result and Disscussion

The dual effect of purified exopolysaccharides of pseudomonas isolates Pseu-7, Pseu-18, Pseu-26, and Pseu-37 and ISR inducing chemicals salicylic acid, jasmonic acid, and azibenzolar on Pyricularia oryzae growth and disease incidence in rice was investigated *in vitro* (Table 1). The study clearly demonstrated that these chemicals have no phytostimulatory activity. The current study's findings also suggested that Pseudomonas exopolysaccharides have a dual effect on host plant growth as well as disease incidence reduction, whereas ISR inducing chemicals only reduced blast disease incidencePhytostimulatory effect of pseudomonas exopolysaccharides on wheat was reported by Bahat-Samet *et al*. (2004) [1]. The current study's findings clearly demonstrated the dual effect (phytostimulatory and biocontrol) of Pseudomonas exopolysaccharides, which is consistent with the earlier findings of Bahat – Samet *et al* (2004) [1]. The effect of exopolysaccharides from pseudomonas isolates Pseu-7, Pseu-18, Pseu-26, and Pseu-37 at different concentrations, namely 100, 200, and 300 ppm, on the incidence of blast disease in rice was studied *in vitro* (Table – 2).

Table 1: Effect of *pseudomonas Exopolysaccharides* (EPS) and ISR inducing chemicals on the enhancement of growth and blast disease incidence (*Pyricularia oryzae*) in rice.

Treatment	Plant Height(cm)***	Disease incidence (%) a,b
Control	13.22±1.00	81.50±1.20
ISR Inducing chemicals **	-	
Salicylic acid	14.00±0.30	20.11±0.44
Jasmonic acid	14.11±0.43	20.42±0.11
Azibenzolar	14.00±0.15	21.12±0.32
Purified Exopolysaccharides (Pseu-7)*	22.12±0.10.	19.79±0.13
Purified Exopolysaccharides (Pseu-18)*	20.06±0.30	18.79±0.38
Purified Exopolysaccharides (Pseu-26)*	22.15±0.41	20.00±0.17
Purified Exopolysaccharides (Pseu-37)*	24.13±0.35	19.9±0.15

Exopolysaccharides collected from minimal medium of Neyra and Van Berkum (1977) [6] supplemented with 0.1% pectic acid and 0.005% after KNO3 after 48 hr of incubation. Purified exopolysaccharides was Prepared according to Kyungseok *et al*. (2008) [5].

** at 0.01 per cent

*** 20th DAS

a. Estimated disease incidence 7 days after Pyricularia oryzae challenge inoculation
b. SD is the standard deviation of the mean of three replications.

Table 2: Methods of Application of *Pseudomonas exopolysaccharides* at different concentrations on Rise blast disease (*Pyricularia oryzae*)

Treatment	Concentration of Exopolysaccharides (ppm)	Disease incidence*a (%)	Statistics b,c
Control	---	78.8±1.05	-
	100	17.2±0.41	e
Purified Exopolysaccharides from (Pseu-7)	200	16.2±0.33	f
	300	16.0±0.10	f
	100	20.2±0.17	a
Purified Exopolysaccharides (Pseu-18)	200	19.3±0.13	b
	300	19.0±0.14	b
	100	18.6±0.37	c
Purified Exopolysaccharides from (Pseu-26)	200	17.7±0.20	d
	300	17.5±0.44	d
	100	15.5±0.14	g
Purified Exopolysaccharides form (Pseu-37)	200	14.4±0.22	h
	300	14.2±0.41	h

* Exopolysaccharides were extracted from the minimal medium of Neyra and van Berkum (1977) [6] supplemented with 0.1 percent pectic acid and 0.005 percent after KNO3, and purified exopolysaccharides were prepared according to Kyungseok *et al* (2008) [5].

A Disease incidence is estimated 7 days after inoculation with Pyricularia oryzae b. The student't' test shows that values followed by different letters differ significantly at the 5% level. The figures represent the average of three replications plus the standard deviation. Purified exopolysaccharides collected from each pseudomonas isolate were found to reduce blast disease incidence in rice to a higher level when compared to control plants. Purified WPS at 200 ppm reduced blast disease incidence to a level comparable to exopolysaccharide application at 300 ppm among the various exopolysaccharide concentrations tested. However, there was a significant difference in blast disease resistance in rice when exopolysaccharide levels were applied at 100 and 200 ppm. The study clearly demonstrated the importance of using 200 ppm pseudomonas exopolysaccharides to effectively reduce blast disease incidence in rice. Kyungseok *et al*. (2008) [5] determined that 200 ppm of purified exopolysaccharides of Burkholderia gladioli IN-26 was the most effective concentration for Colletotrichum orbiculare biocontrol in cucumber. However, no previous reports on the biocontrol effect of Pseudomonas exopolysaccharides at various concentrations against P.oryzae have been published. More research is needed to optimise Pseudomonas exopolysaccharides (200 ppm) for effective biocontrol of Pyricularia oryzae incidence in rice.

References

1. Bahat Samet, Castro Sowinski ES, Okon Y. Arabinose content of extra cellular polysaccnaride plays a role in cell aggregation of Azospirillum brasilence FEMS Microbill. Lett,2004:237:195-203.
2. Day JM, Dobereiner J. Physiological aspects of N2 fixation by a Spirillum from Digitaria roots. Soll Biol. Biochem,1976:8:45-50.
3. Gomez KA, Gomez AA. Statistical procedures for agricultural research, John Wiley and Sons, New York, USA, 1984.
4. IRRI. International Rice Research Instityte. Annual Report for 1979. Los Banos, Philippines, 1980, 171-182.
5. Kyungseok P, Kloepper JW, Ryu CM, 2008.
6. Neyra CA, Van Berkum P. Nitrogen fixation grasses, Advan, Agron,1977:29:37-38.