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EPS Rich floccugated culture of *Pseudomonas* for the enhancement of growth and effective Biocontrol against *Pyricularia oryzae* of Rice Var. (ADT-36)

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Abstract

A study was conducted by using different concentrations of purified *exopolysaccharides* (EPS) *viz.,* 100, 200, 300 ppm on the blast disease incidence of rice revealed that the application of the same at 200 ppm concentration could effectively controlled the disease incidence to a higher level when compared to other concentration. The results also reflected the dual effect of *Pseudomonas* exopolysaccharides on the enhancement of host plant growth as well as the bio-control against *Pyricularia oryzae* whereas the application of Induced Systemic Resistance inducing chemicals confined with reduction in blast disease incidence. Interestingly the *Pseudomonas* exopolysaccharides at a concentration of 200 ppm level could be optimized as effective one for the control of blast disease in lowland rice.

Key words : *Pseudomonas exopolysaccharides* (EPS), Bio-control of blast disease.

In India, rice (*Oryza sativa* L.) is grown under both upland and lowland conditions and out of 44 million hectare of rice cultivated area, 12 per cent of the same grown under rainfed upland condition. The blast disease, caused by *Pyricularia oryzae*, is one of the most destructive fungal diseases of lowland rice crop, causing an yield loss up to 90 percent. PGPR mediated (ISR) against blast pathogen seems to be a positive approach in the reduction of biological and environmental hazards posed by the application of synthetic chemical pesticides.

Neyra *et al*.,[6] proposed the use of "exopolysaccharides mediated *Pseudomonas* as a delivery system, for the enhancement of growth and yield of crop plants under stress condition, including, moisture and temperature. Even though many reports suggested the positive role of pseudomonas inoculation in *rice crop*, the role of exopolysaccharides – *rich flocculated* culture of pseudomonas application on the induction of systemic resistance (ISR) against *Pyricularia oryzae* in lowland rice has not been studied, so far. The present study has been undertaken with an aim to elucidate the role of *Pseudomonas exopolysaccharides,* as

an elicitor, on the induction of (ISR) against *Pyricularia oryzae* in lowland rice crop.

Preparation Pseudomonas strains :

Four efficient *Pseudomonas* strains *viz.,* Pseu-7, Pseu-18, Pseu-26, Pseu-37, strains from the rhizosphere of lowland rice var. ADT-36, were used. All the *Pseudomonas* isolates were grown separately in synthetic malate broth[2] supplemented with 0.05 per cent yeast extract (W/V) in a shaking bath at 30 ± 20°C for 24hrs. Then, the medium was centrifuged at 5000 x g for 10 min to harvest the log phase cells and the pellets washed three times with 0.1 M phosphate buffer (pH 6.8), finally, the cells were resuspended in the same buffer to a cell concentration of 1 x 10^9 CFU/mL by measuring the OD at 420 nm and used as inoculums.

Pseudomonas exopolysaccharides (EPS):

Minimal salts medium[6] was used for the present study together with addition of 8mM fructose and 0.5mM KNO_3, as sole carbon and nitrogen source. Each 1 ml culture of *Pseudomonas* strains (1 x 10^{-7} CFU/ml) was added to 100ml of fructose medium dispensed in 250ml Erlenmeyer flask and incubated at 30 ± 2°C for 5 days under shaking condition (250rpm) in rotary shaker.

After the incubation period, the Exopolysaccharides produced by individual *Pseudomonas* strains were extracted and purified according to Kyungseok *et al.,*[5] and used at different concentrations *viz.,* 100, 200 and 300 ppm.

Preparation of induced systemic Resistance chemicals :

ISR chemicals *viz.,* salicylic acid, jasmonic acid and *Pseudomonas* (Himedia, India) at a level of 0.01 per cent concentration were used.

Treatments :

The following treatments *viz.,* ISR inducing chemicals at 0.01 percentage concentration and *Pseudomonas* exopolysaccharides at 200 ppm concentration were used for assessing the biocontrol ability against *Pyricularia oryzae,* whereas, the optimization of different concentration of *Pseudomonas exopolysaccharides* on the blast disease incidence was tested at 100, 200 and 300 ppm concentration levels.

Preparation of growth chamber :

The growth chamber was a desiccators (12 x 10 cm) consisting of two parts. The lower part was filled with Weaver's medium and upper part contained stainless steel wire mesh (mesh size 3 mm) supports. The lid was placed over the cotton and the chamber was closed before sterilization. The growth chamber was sterilized by autoclaving. After the sterilization of growth chamber, fifty germinated rice seeds with coleoptiles (2 cm high) were transferred aseptically onto the stainless steel wire mesh and incubated for 10 days. The growth chamber was maintained under 14 hrs day and 10 hrs night cycle and the temperature ranging from 24°C at night to 32°C around noon. By this time, the rice roots yielded many lateral roots, well spread in the Weaver's medium maintained at the lower part of the growth chamber.

Inoculation of rice crop with Pyricularia oryzae :

P. oryzae AU–1 (provided by Department of plant pathology, Annamalai university) was maintained in oat meal agar (OMA) medium and used for the challenge inoculation purpose. Thick spore suspension of the same was prepared with sterile distilled water from 10 days old culture maintained in OMA medium and strained through double layer muslin cloth so as to get a free suspension of conidia. The population was adjusted with the help of Haemocytometer and a spore suspension with optimum spore concentration (50,000 spore'sml-1) was prepared. Then, the spore suspension was added with few drops of Tween – 80 which increased the adherence capacity of the spores and acts as a sticker. The spraying of spore suspension was done under proper humid condition. Control plants were also sprayed with sterile distilled water. After one week of challenge inoculation the blast disease incidence was enumerated with a score chart of 0-9 grades devised by International Rice Research Institute[4]. The statistical analysis was carried out according to Gomez and Gomez[3].

The dual effect of the purified exopolysaccharides of *Pseudomonas* isolates *viz.,* Pseu-7, Pseu-18, Pseu-26, and Pseu-37 and ISR inducing chemicals, namely, salicylic acid, jasmonic acid and azibenzolar on the growth and *Pyricularia oryzae* disease incidence in rice was studied under *in vitro* condition (Table-1).

The study clearly revealed the absence of phytostimulatory activities of these chemicals. The results of the present study also suggested the dual effect of *Pseudomonas exopolysaccharides* on the augmentation of growth of the host plant as well as the reduction in disease incidence. Whereas, the ISR inducing chemicals confined with reduction in blast disease incidence alone. Bahat-Samet *et al.,*[1] reported the phytostimulatory effect of *Pseudomonas exopolysaccharides* on wheat. The results of the present study clearly

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	13.20±1.00	80.49±1.19
ISR Inducing chemicals **	-	
Salicylic acid	14.00±0.32	19.10±0.43
Jasmonic acid	14.10±0.41	20.41±0.10
Azibenzolar	14.00±0.14	20.11±0.31
Purified *exopolysaccharides* (Pseu-7)*	21.11±0.9	19.78±0.12
Purified *exopolysaccharides* (Pseu-18)*	19.05±0.32	18.77±0.37
Purified *exopolysaccharides* (Pseu-26)*	21.14±0.40	20.00±0.16
Purified *exopolysaccharides* (Pseu-37)*	23.12±0.34	18.9±0.14

revealed the dual effect (Phytostimulatory and biocontrol) of *Pseudomonas exopolysaccharides* and in conformity with the earlier findings of Bahat – Samet *et al*.,[1].

The effect of *exopolysaccharides* of *Pseudomonas* isolates *viz.,* Pseu-7, Pseu-18, Pseu-26 and Pseu-37 at different concentrations, *viz.,* 100, 200 and 300 ppm on the blast disease incidence of rice was studied under *in vitro* condition (Table – 2).

Exopolysaccharides collected from minimal medium of Neyra and Van Berkum[6] supplemented with 0.1% pectic acid and 0.005% after KNO_3 after 48 hr of incubation. Purified *exopolysaccharides* was prepared according to Kyungseok *et al*.,[5].

** at 0.01 per cent
*** 20th DAS

a. Disease incidence estimated 7 days after challenge inoculation with *Pyricularia oryza*e
b. Values are mean of three replications ± SD

Table-2. Methods of Application of *Pseudomonas exopolysaccharides* at different concentrations on Rice blast disease (*Pyricularia oryzae*)

Treatment	Concentration of *exopolysaccharides* (ppm)	Disease incidence*a (%)	Statistics b,c
Control	---	77.7±1.04	-
	100	17.1±0.40	e
Purified *exopolysaccharides* from (Pseu-7)	200	16.1±0.32	f
	300	16.0±0.11	f
	100	20.1±0.16	a
Purified *exopolysaccharides* (Pseu-18)	200	19.2±0.12	b
	300	19.0±0.11	b
	100	18.5±0.36	c
Purified *exopolysaccharides* from (Pseu-26)	200	17.6±0.19	d
	300	17.4±0.43	d
	100	15.4±0.13	g
Purified *exopolysaccharides* form (Pseu-37)	200	14.3±0.21	h
	300	14.1±0.40	h

* *exopolysaccharides* collected from minimal medium of Neyra and van Berkum[6] supplemented with 0.1% pectic acid and 0.005% after KNO_3 after 48 hr of incubation and Purified *exopolysaccharides* was prepared according to Kyungseok *et al*.,[5].

a. Disease incidence estimated 7 days after challenge inoculation with *Pyricularia oryzae*
b. Values followed by different letters are significantly differed at 5 % level according to student 't' test c. Values are mean of three replications ± SD.

It was found that the application of purified *exopolysaccharides,* collected from each *Pseudomonas* isolate, was found to reduce the blast disease incidence in rice to a higher level when compared to control plants, Among the different concentrations of *exopolysaccharides,* the application of purified WPS at 200 ppm level reduced the blast disease incidence to a level on par with 300 ppm level of *exopolysaccharides* application. However, a marked variation was observed between 100 and 200 ppm level of exopolysaccharides application regarding the blast disease resistance in rice. The study clearly revealed that the importance of *Pseudomonas exopolysaccharides* application at 200 ppm level on the effective reduction of blast disease incidence in rice.

Kyungseok *et.al.,*[5] studied that the different concentration of purified *exopolysaccharides of Burkholderia gladioli* IN-26 against *Colletotrichum orbiculare* and optimized 200 ppm as effective concentration for the biocontrol of *Colletotrichum orbiculare* in cucumber. However, there were no earlier reports regarding the biocontrol effect of *Pseudomonas exopolysaccharides* at different concentrations, available against *P.oryzae.* The optimization of *Pseudomonas exopolysaccharides* (200 ppm) for the effective biocontrol of *Pyricularia oryzae* incidence in rice and the subject needs further elaborate research.

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