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RESEARCH ARTICLE

STUDIES ON THE EFFECT OF PGPR INOCULATED CELLS ON THE MAXIMIZATION OF ISR MEDIATED, RICE PATHOSYSTEM IN CAUVERY DELTAIC REGIONS OF TAMIL NADU

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ABSTRACT

The comparative evaluation of different bioformulations, viz., vegetative cell application, co-inoculation and co-aggregates application of efficient PGPR cells viz., *Pseudomonas fluorescens* (PF.3) and *Bacillus polymyxa* (B.19), together with challenge inoculation of *Pyricularia oryzae* on the enhancement of induced systemic resistance (ISR) in Rice. *Pyricularia oryzae pathosystem* was studied under pot culture condition with rice cv. ASD.19. It was observed that the application of *Pseudomonas fluorescens and Bacillus polymyxa*, as coaggregates, altered the biochemical and physiological parameters viz., reducing and nonreducing sugars, total phenol content and defense enzymes activities such as perocidase (PO), *polyphenol oxidase* (PPO), of rice plant to a significant livel followed by co inoculation and vegetative cell application of PGPR cells. The application of PGPR cells, as coaggregates, was found to augment the total phenol content and defense enzyme activities such as PO and PPO content of rice plant to a higher level whereas a reduction in reducing and nonreducing sugar level was recorded, which ultimately lead to a reduction of *Pyricularia oryzae* incidence in upland rice. It has been postulated that the EPS biosynthesis of PGPR cells during co aggregation processes might act as elicitor for the enhancement of ISR in Rice *Pyricularia oryzae pathosystem* whereas the vegetative cells and coinoculation processes, without any involvement of EPS, responded poorly for the enhancement of ISR in the same *pathosystem*.

INTRODUCTION

Rice (*Oryza sativa D* is the foremost cereal of the world and is the staple food of more that 60% of the world's population. In India, rice is cultivated under irrigated lowland. Rainfed lowland, rainfed upland and deep water systems. Among the different rice production systems of India. The rainfed upland econlogy is the first and foremost one in terms of area and production, but normally with least productivity. Of the several biotic and abiotic constraints, low soil fertility and incidence of diseases are considered to be the major constraints that eventually lead to the low productivity in upland rice. Hence, the upland rice productivity must be greatly increased by providing additional nutrient inputs and through effective control of phytopathogens.

Moreover, the incidence of blast disease caused by *Pyricularia oryaze, one of the most destructive fungal disease of* upland rice crop, causing an yield loss upto 90 percent. The use of plant growth promoting rhizobacteria (PGPR) as a biological approach, might be an alternative strategy to overcome the biological and environmental hazards posed by the persistent use of synthetic chemicals. Rhizosphere bacteria that favourably affect the plant growth and yield of commercially important crops are denominated as "Plant growth promoting rhizobacteria (PGPR)" (Kloepper *et al.,* 1980). Several mechanisms of plant microbe interaction may participate in the association and affect plant growth, including N* fixation, hormonal interaction, improvement in root growth, solubilisation of nutrients and biocontrol against phytopathogens. Thus, the PGPR affect the plant growth directly by producing and secreting plant growth promoting substances or by eliciting root metabolic activities by supplying biologically fixed nitrogen and indirectly by acting against phytopathogenic microorganisms (Kloepper *et al*., 1989). The well known PGPR include, bacteria belonging to the genera, namely, *Azoarcus, Klebsiella, Arthrobacter, Enterobacter, Serratia* and *Rhizobium*. Fluorescent *Pseudomonas* has emerged as the largest, potentially most promising group of PGPR, possessing traits also involved in the biocontrol of phytopathogens, due to the production of secondary metabolites such as siderophore, antibiotics and phytohormone production for plant growth development (Suslow, 1982; Kloepper *et al*., 1980) *bacillus polynyxa (Bacillus polymyxz;* Ash *et al.,* 1994) a common soil bacterium belongs to the group of plant growth promoting rhizobacteriz (PGPR) (Timmusk *et al*., 1999; Selim *et al*., 2005). The PGPR

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characteristics of *bacillus polymyxa have been frequently reported by* (Gjung Kahng *et al*., 2001).

Thus the present work was aimed to investigate the effect of different PGPR on the enhancement of ISR in Rice *Pyricularia oryzae pathosystem under upland condition.*

MATERIALS AND METHODS

A pot culture experiment was conducted to study the effect of different formulations of PGPR cells viz., single strain inoculation, coinoculation and coaggregates application together with challenge inoculation of *pyricularia oryzae on the enhancement of growth and* yield in upland rice with special emphasis to ISR mediated *biocontrol* against blast disease (*Pyricularia oryzae).* The study was conducted with rice cultivar ASD-19 at the *polyhouse* of Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, India.

Rectangular cement pots with 18" x 12" x 12" size were filled with 45kg of paddy field soil flooded with water for 2 days and brought to fine puddle condition. Seeds of the rice variety ASD. 19 were loosely packed separately in small gunny bag and soaked in water for 12 hr. Then, the bags were subsequently kept in dark place after covering with wet gunny bags to ensure optimum condition for germination. The seeds germinated within 24hrs. After soaking. The pre-germinated seeds of rice (cv., ASD 19) was sown in rows in pots separately, On the 5th day of sowing, the seedlings were thinned to get 50 numbers per pot. The age of the seedlings were counted from the time of sowing. The experiment was arranged in randomized block design (RBD) with three replications and the following were the treatments. T_1. Control, T_2 *Pseudomonas fluorescens* alone T_3. *Bacillus Polymyxa alone,* T_4 *Pseudomonas fluorescens + Pseudomonas fluaresoens + Bacillus Polymyxa cpaggregates* application. During the experimental period, the annual mean minimum and the maximum temperature of experimental area is 25^{o}C and 30^{o}C, respectively and the mean highest and lowest humidity were 96 and 78 *percen*, respectively. The mean annual rain fall of this area is 1500 mm.

A fertilizer schedule of 100; 50; 50 NPK ha^{-1} was followed, Regarding the 'N' fertilization, 50 per cent of the same was given as basal dose, while the other 50 per cent was given as top dressing in two split doses. The entire dose of P_2O_3 and K_2O has been applied basally as super phosphate and muriate of potash, respectively.

Rice plants were challenge inoculated by spraying the *P. oryzae spore suspension at (50,000 spore/ ml inoculums level) on 10th DAS with an atomizer and the control plant was sprayed with sterile water high humidity was created by sprinkling the water frequently in the polyhouse. The crop was given a hand weeding on 30th DAS and well protected against pests and diseases. The experiment was maintained under limited water supply as per the conditions prevailing in upland rice ecosystem. Five representative samples of plant hills in each pot were pegmarked for periodical observations.*

The plant height, shoot dry weight, root dry weight, chlorophyll content (Mahadevan and Sridhar, 1986), IAA production (Tien *et al*., 1979), phosphorous content (Watanable and Oslen 1965), grain and straw yield of upland rice wsas recorded on 45th DAS. The reducing and non-reducing sugar was estimated according to (Mahadevan and Sridhar, 1986) whereas, the total phenol content was assayed according to (Malik *et al*., 1997). The defense enzyme activities such as peroxidase (PO), Polyphenol oxidase (PPO) was assayed according to Putter, (1974) and Ester Bauer, (1977) respectively.

RESULTS AND DISCUSSION

The effect of different bioformulations viz., single strain inoculastion, coinoculation and coaggregates application of PGPR cells. Viz., *pseudomonas fluorescens and bacillus polymyxa* on the growth yield parameters viz., plant height, root and shoot dry weight, phosphorus, IAA and chlorophy content, grain and straw yield of upland rice cv. ASD.19 was studied under pot culture condition (Table 1). It was observed that all the formulations of PGPR cells could augment the growth and yield parameters of upland rice cv. ASD.19 when compared to control (without bioinoculation). These observations clearly revealed the positive effect of PGPR cells inoculation in augmenting the growth and yield parameters of upland rice.

Table 1. Effect of different formulations of PGPR cells on the enhancement of growth and yield parameters in Upland rice (*Oryza sativa) cv*

ASD – 19

Treatment	Plant height (cm)	Roodt dry weight (g/plant)	Shootdry weight (g/plant)	Phosphorous content (%)	Chlorophy II content (mg/g of leaf)	IAA content (%)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Control	52.09	0.270	1.043e	0.42	2.36e	10.78e	5.54e	…..
Pseudomonas flouorescens alone	62.35e	0.314e	1.240e	0.65e	2.35e	12.42e	5.70e	10.32
Bacillus polymyxa alone	60.67d	0.30d	1.172d	0.56d	2.32d	11.80d	5.55d	*9.67*
Pseudomonas fluorescens+ Bacillus polymyxa co inoculation	65.10b	0.331b	1.356b	0.72b	2.65b	14.02b	5.82b	13.84
Pseudomonas fluorescens+Bacillus Polymyxa coa	68.75a	0.335a	1.486a	0.84a	2.86a	16.24a	5.95	15.38
LSD (P = .05)		0.008	0.182	0.09	0.12	0.15	0.03	

a Average of three replication b Values followed by different letters are significantly differed at 5% level according to student 't' test

Regarding the different formulations of PGPR cells, the application of "Intergeneric PGPR coaggregates" consisting of *Pseudomonas fluorescens and bacillus polymyxa* could augment the frowth and yield parameters of upland rice to a

higher level followed by coinoculation and single strain inoculation treatments viz., Pseudomonas fluorescens *alone and bacillus polymyxa alone, the inoculation of Pseudomonas fluorescens* alone treatemtn recorded the higher value for the above parameters than *bacillus ploymyxa alone treatment. The individual inoculation effect* of *Pseudomonas* and *bacillus in augmenting the growth and yield parameters of rice has* already been reported (Guemouri – Athmani *et al*., 2000; Vonderweid *et al*., 2000).

The positive coinoculation effect of Pseudomonas and *Bacillus has* already been reported by EL – Komy *et al*. (2004) in wheat, Neyra *et al*. (1999) reported the positive effect of *Azospirillum and Rhizobium* coflocs on the enhancement of growth and yield in common bean. Greater plant height of rice been reported by Agarwal and Singh (2000). Increase in dry grain and straw yield of rice has been reported by (Ding *et al.,* 2005; Selvakumari *et al.,* 2000; Nadeem *et al.,* 2006). However, the application effect of PGPR coaggregates viz., *Pseudomonas and bacillus to rice crop has not been reported, so far, this is the first comprehensive report regarding the positive role* of PGPR coaggregates in augmenting the growth and yield parameters in upland rice. The studies on the effect of different Bioformulations of PGPR cell on the enhancement of ISR mediates Biocontrol of *P. Oryzae with special* Emphasis to biochemical and physiological aspects, revealed the highest performance of PGPR coaggregates in augmenting the phenol metabolism viz., total phenol content and orthodihyroxy phenol, carbohydrate metabolism viz., reducing and non reducing sugar level and defense enzyme activities viz., Peroxidase (PO) and Polyphenoloxidase (PPO) of upland rice plant followed by co* inoculation of PGPR cells, *Pseucomonas fluorescens alone and bacillus Polymyxa* alone treatments (Fig 1 to Fig 6). The application of PGPR voaggregates consisting of *Pasedomonas and bacillus sp* augmented the total phenol, OD phenol PO and PPo activities of upland rice plant to a higher level whereas a reduction in reducing and nonreducing sugar levels, observed. Farkas and Kiraley (1962) correlated the increasing levels of phenol contents of host plant with resistance to phytopathogens. It is well known that OD phenols are the most active forms of phenol and their oxidation products are more toxic than phenol. The oxidation is mediated by the enzme PO and PPO and the resulting quinines are effective inhibitors of SH group of enzymes which may be inhibiting to the pathogens (Goodman *et al*., 1967). Usharnai, (2005) reported the induction of phenolics content of rice plant due to *Pseudomonasinoculation* and challenge inoculation of *P. Oryzae.* Mishra *et al.* (2006) reported the *Rhizobium* mediated induction of phenolics in rice plant during the challenge inoculation of *P. Oryzae.* Nanthakumar (1998) correlated the ISR with two fold increase in peroxidase activity against rice sheath pathosystem *(Rhizoctonia solami)* in rice plant. As a major source of energy, the incidence and development of disease. Plant tissues containing greater reserves of oxidisable carbohydrates are oftern more prone to pathogenic invasion that tissues containg low reserves. Altered carbohydrate metabolism of host plant in response to pathosystem infection was studied by several workers (Bhaskaran and Prasad 1971; Kalyanasundaram 1986). The sugar content in healthy and pathogen inoculated plants was very often correlated with resistance mechanism (Horsfal and Diamond 1957). In the present study also, the reducing and non reducing sugar levels were found to decrease with PGPR coaggregates application together with challenge inoculation of *P. Oryzae.* The higher rate of reduction in the native level of reducing sugars, may be one among the vital phenomena contributing resistance to plant. The results of present study clearly envisaged the positive role of PGPR consisting of *Pseudomonas and Bacillus* isolates in augumenting the ISR against *P. Oryzae* in upland rice crop. However, the mechanism of PGPR coaggregates mediated ISR against *P. Oryzae* in rice plant is still unclear and the subject needs further elaborate research

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