CURRENT BOTANY

Curr. Bot. 2(3):12-17, 2011

REGULAR ARTICLE

Comparative evaluation of different bioformulations of PGPR cells on the enhancement of induced systemic resistance (ISR) in Rice *P. oryzae* pathosystem under upland condition

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KEYWORDS

PGPR, Bio-formulation, Rice, Pyricularia oryzae, ISR

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EDITOR
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CB Volume 2, Year 2011, Pages 12-17

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 Paenibacillus 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 Paenibacillus polymyxa, as co-aggregates, altered the biochemical and physiological parameters viz., reducing and non-reducing sugars, total phenol content and defense enzymes activities such as peroxidase (PO), polyphenol oxidase (PPO), of rice plant to a significant level followed by co-inoculation and vegetative cell application of PGPR cells.

The application of PGPR cells, as co-aggregates, 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 non-reducing 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 co-inoculation processes, without any involvement of EPS, responded poorly for the enhancement of ISR in the same pathosystem.

This is the first comprehensive report on the role of bacterial EPS, as a determinant of ISR, in Rice - Pyricularia oryzae pathosytem and it needs further elaborate research on the topic.

Introduction

Rice (*Oryza sativa L*) is the foremost cereal of the world and is the staple food of more than 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 ecology 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.

Phosphorous, is one of the essential nutrients, required for the growth of both plants and microorganisms. Phosphorous is generally available in the form of insoluble calcium phosphate and the fixation of the same leads to reduction in biological nitrogen fixation. Moreover, the incidence of blast disease caused by *Pyricularia oryzae,* 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, *Azospirillum, Azotobacter, Pseudomonas, Bacillus, Azoarcus, Klebsiella, Arthrobacter, Enterobacter, Serratia* and *Rhizobium* on non-legumes.

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, antibotics and phytohormone production for plant growth development (Suslow, 1982; Kloepper *et al.,* 1980).*Paenibacillus*

polymyxa (*Bacillus polymyxa*; Ash *et al.,* 1994) a common soil bacterium belongs to the group of plant growth promoting rhizobacteria (PGPR) (Timmusk *et al.,* 1999; Selim *et al.*, 2005). The PGPR characteristics of *Paenibacillus polymyxa* have been frequently reported by (Gjung Kahng *et al.*, 2001).

Thus the present work was aimed to investigate the effect of different bioformulations of PGPR cells 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, co-inoculation and co-aggregates 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 during Rabi season (Sep to Jan, 2009-10) 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"x12"x12" size were filled with 45 kg 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 24 hr. 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 - *Paenibacillus polymyxa* alone, T_4 - *Pseudomonas fluorescens* +*Paenibacillus polymyxa* co-inoculation and T_5-*Pseudomonas fluorescens*+ *Paenibacillus polymyxa* co-aggregates application.

During the experimental period, the annual mean minimum and the maximum temperature of experimental area is 25ºC and 39ºC, respectively and the mean highest and lowest humidity were 96 and 78 percent, 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_5 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 inoculum 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 was 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 inoculation, co-inoculation and co-aggregates application of PGPR cells *viz., Pseudomonas fluorescens* and *Paenibacillus polymyxa* on the growth yield parameters *viz.,* plant height, root and shoot dry weight, phosphorus, IAA and chlorophyll 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. Regarding the different formulations of PGPR cells, the application of "Intergeneric PGPR co-aggregates" consisting of *Pseudomonas fluorescens* and *Paenibacillus polymyxa* could augment the growth and yield parameters of upland rice to a higher level followed by co-inoculation and single strain inoculation of PGPR cells between the two single strain inoculation treatments *viz., Pseudomonas fluorescens* alone and *Paenibacillus polymyxa* alone, the inoculation of *Pseudomonas fluorescens* alone treatment recorded the higher value for the above parameters than *Paenibacillus ploymyxa* alone treatment. The individual inoculation effect of *Pseudomonas* and *Paenibacillus* in augmenting the growth and yield parameters of rice has already been reported (Guemouri-Athmani *et al.*, 2000; Vonderweid *et al.*, 2000). The positive co-inoculation 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 due to the inoculation of *Pseudomonas* and *Paenibacillus* has been reported by Agarwal and Singh, (2000). Increase in dry matter production, phosphorus content, chlorophyll content, 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 co-aggregates *viz., Pseudomonas* and *Paenibacillus* to rice crop has not been reported, so far. This is the first comprehensive report regarding the positive role of PGPR co-aggregates in augmenting the growth and yield parameters in upland rice.

The studies on the effect of different bioformulations of PGPR cells on the enhancement of ISR mediates biocontrol of *P.oryzae* with special emphasis to biochemical and physiological aspects, revealed the highest performance of PGPR co-aggregates 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, *Pseudomonas fluorescens* alone and *Paenibacillus polymyxa* alone treatments (Fig1toFig6). The application of PGPR co-aggregates consisting of *Pseudomonas* and *Paenibacillus* 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 non-reducing 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 enzyme 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). Usharani, (2005) reported the induction of phenolics content of rice plant due to *Pseudomonas* inoculation 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 solani*) in rice plant. As a major source of energy, the level of carbohydrates of host plant has great influence on the incidence and development of disease. Plant tissues containing greater reserves of oxidisable carbohydrates are often more prone to pathogenic invasion than 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 co-aggregates 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 *Paenibacillus* isolates in augumenting the ISR against *P.oryzae* in upland rice crop. However, the mechanism of PGPR co-aggregates mediated ISR against *P.oryzae* in rice plant is still unclear and the subject needs further elaborate research

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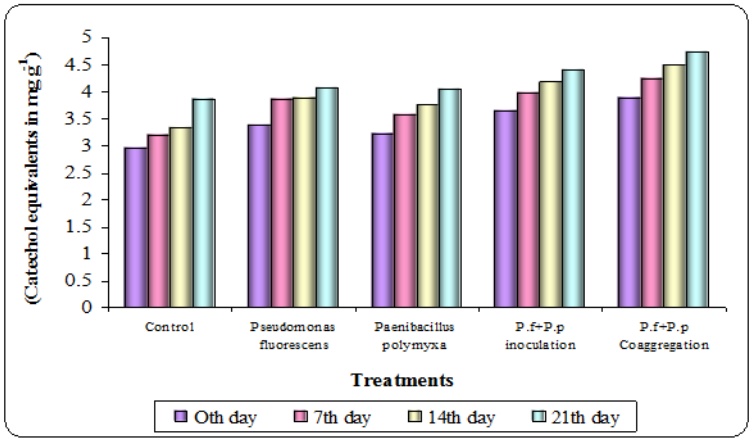

Figure – 1: Changes in total phenol content of BPT-5804 rice as influenced by the application of different formulations of PGPR cells during the challenge inoculation of *Pyricularia oryzae*

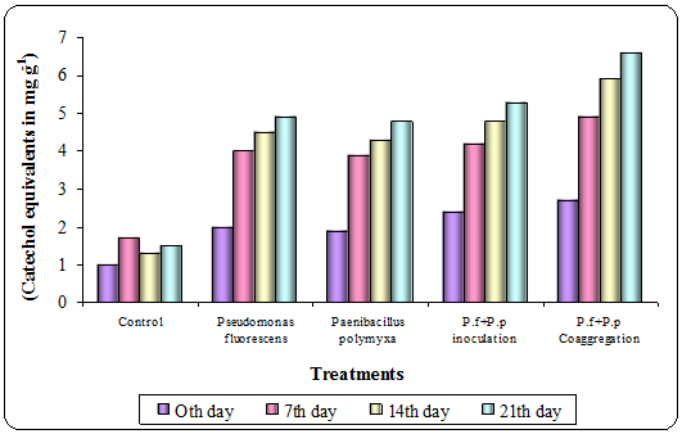

Figure – 2: Changes in Ortho-dihydroxy phenol (OD phenol) content of BPT-5804 rice as influenced by the application of different formulations of PGPR cells during the challenge inoculation of *Pyricularia oryzae*

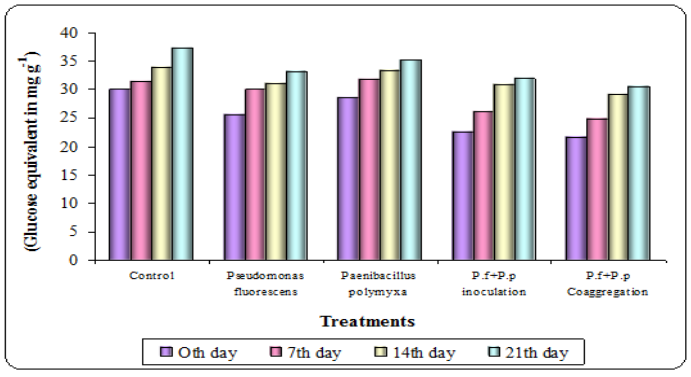

Figure – 3: Changes in Reducing sugar content of BPT-5804 rice as influenced by the application of different formulations of PGPR cells during the challenge inoculation of *Pyricularia oryzae*

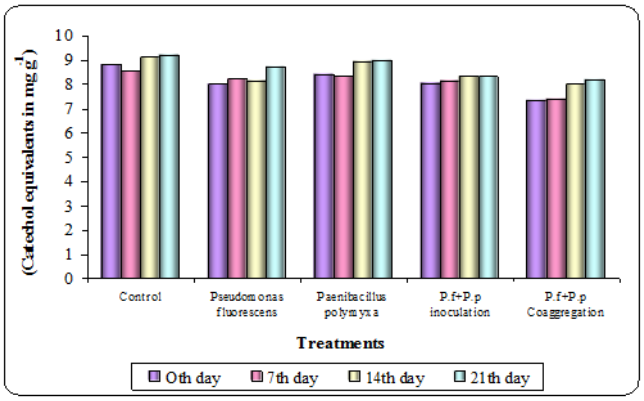

Figure – 4: Changes in Non-reducing sugar content of BPT-5804 rice as influenced by the application of different formulations of PGPR cells during the challenge inoculation of *Pyricularia oryzae*

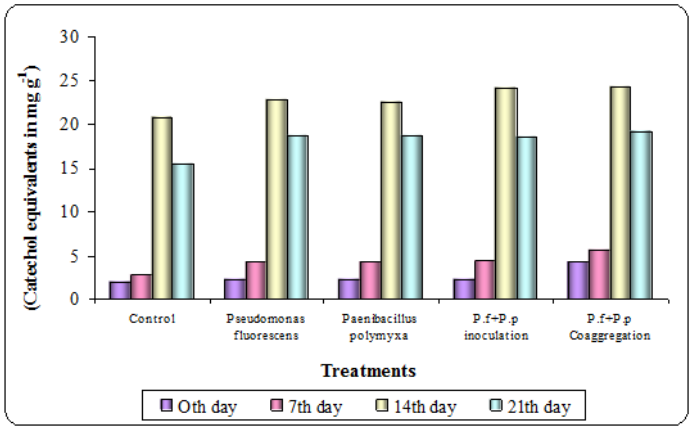

Figure – 5: Changes in Polyplhenol oxidase content of BPT-5804 rice as influenced by the application of different formulations of PGPR cells during the challenge inoculation of *Pyricularia oryzae*

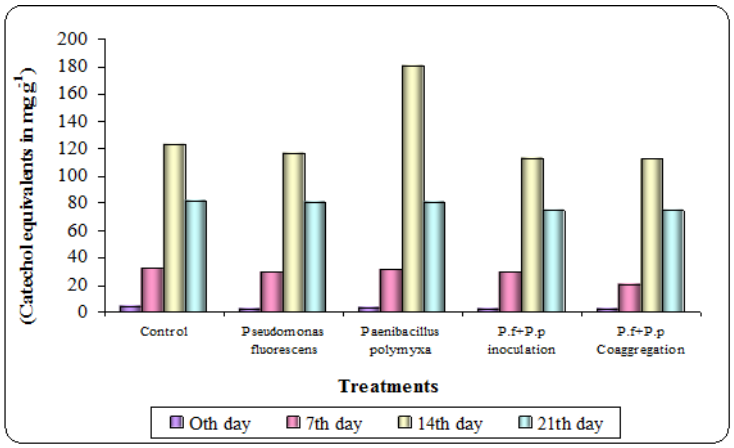

Figure – 6: Changes in Peroxidase content of BPT-5804 rice as influenced by the application of different formulations of PGPR cells during the challenge inoculation of *Pyricularia oryzae*

Table-1: Effect of different formulations of PGPR cells on the enhancement of growth and yield parameters in Upland rice (*Oryza sativa*) cv. BPT-5804

Treatment [a]	Plant height (cm)	Root dry weight (g/plant)	Shoot dry weight (g/plant)	Phosphorous content (%)	Chlorophyll content (mg/g of leaf)	IAA content (%)	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Control	52.10^{e}	0.270^{e}	1.043^{e}	0.44^{e}	2.36^{e}	10.78^{e}	5.54^{e}	----
Pseudomonas fluorescens alone	62.36^{c}	0.315^{c}	1.241^{c}	0.67^{c}	2.58^{c}	12.43^{c}	5.71^{c}	10.32
Paenibacillus polymyxa alone	60.65^{d}	0.307^{d}	1.173^{d}	0.58^{d}	2.84^{d}	11.81^{d}	5.58^{d}	9.67
Pseudomonas fluorescens+ Paenibacillus polymyxa co-inoculation	65.21^{b}	0.332^{b}	1.358^{b}	0.76^{b}	2.69^{b}	14.05^{b}	5.84^{b}	13.84
Pseudomonas fluorescens+ Paenibacillus polymyxa co-aggregates	68.85^{a}	0.339^{a}	1.489^{a}	0.85^{a}	2.87^{a}	16.25^{a}	5.95^{a}	15.38
LSD (P = .05)		0.008	0.182	0.09	0.12	0.45	0.03	-

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

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