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Effect of Bioinoculation silicate solubilizing bacteria in augmenting the plant height and biocontrol of blast Incidence in low land rice

M.Vijiyapriya, S.Mahalakshmi and R.Krishnaveni

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Department of Microbiology, Annamalai University, Annamalai Nagar-608 002.

Abstract

Silicon nutrition and blast disease incidence are the key factors that most frequently limit the productivity of low land rice crop. The present work was carried out to study the bioinoculation effect of different formulations of silicate solubilizing bacteria(SSB) isolate together with organic rice residues supplementation for the augmentation of plant growthand ISR mediated biocontrol against P*yriculariaoryzae* in lowland rice crop. The results revealed that the application of SSB isolate in the form of biofloc (natural) along with organic siliceous material and challenge inoculation of *P. oryzae*augmented the plant height and reduced the plant disease incidence to a maximum level followed by treatment SSB alone.

Key words: Blast disease, SSB-silicate solubilizing bacteria, ISR-Induced systemic resistance, *Pyriculariaoryzae.*

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INTRODUCTION

Blast disease of rice caused by *P.oryzae cav*. is one of the most destructive fungal diseases of rice, causing yield loss upto 90 percent and has ubiquitous occurrence in almost all the rice growing countries including India. The early symptoms of fungal infection arethe formation of grayish to brownish spots or lesions followed by nodal rot and or neck blast which can cause necrosis and frequently resulted in breakage of the panicle (Agarwal and Singh, 2000). Therefore controlling this destructive disease is one of the main goals of the world rice growers (Lucas *et al*.,2009).

Silicon is the second most abundant mineral in soil comprising approximately 28percent of earth's crust (Epstein,1999). Rice is a siliceous plant and a well known silicon accumulator (Takahasi *et al*.,1990) and the crop benefits from silicon nutrition so that silicon fertilization is highly essential for rice crop to maximize the yield. Kim *et al*., (2002) investigated some of the cytological features of Si-mediated resistence to blast disease in rice. Chemical studies on rice blast disease on silicon research reported that there was a definite relationship existing between silicon content of rice plant and blast susceptibility.

Numerous studies showed the effect of SSB on the nutrient uptake from the soil, their positive influence on photosynthesis and the growth of some crops (Han and Lee, 2005; Han *et al*., 2006; Tripti *et al*., 2017). At present, the production of a sufficient number of "ecologically clean" food products is one of the global challenges facing humanity (Dubey and Nidhi Shukla, 2014). To satisfy the demand in food and increase the productivity of crops some non-environmentally friendly technologies are commonly been used such as pesticides and synthetic fertilizers, which can cause health problems. An alternative to this is organic agriculture which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. This is accomplished by using new safe technologies, such as the usage of biofertilizers (Aggani, 2013; Malusa *et al*., 2016). The beneficial effect of silicon application on the components of host disease resistance and concomitant reduction in rice blast severity has been recently reviewed by Rodriguez *et al*., (2005) and numerous mechanisms have been proposed for silicon mediated blast disease resistancethat include

(i) Deposition of silicon beneath the cuticle and act as a physical barrier of fungal pathogen,
(ii) Production ofphenolics and phytoalexins in response to fungal infection, and
(iii) Induction of host defense mechanism.

MATERIALS AND METHODS

Effect of different formulations of silicate solubilizing bacterial cells on incidence of blast disease (*Pyricularia oryzae*) in rice

Rice (*Oryza sativa*) var. IR 50 seeds were surface sterilized, germinated and transferred onto a steel wire

*Corresponding Author :
email: *vijayapriyabalu1978@gmail.com*

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mesh (3 mm dia) in a growth chamber filled with 100 ml weaver's medium. One ml of inoculums of the silicate solubilizing bacterial isolates in different formulations *viz*.,

(i) Vegetative cells of silicate solubilizing bacteria

(ii) Natural biofloc of silicate solubilizing bacteria

(iii) Artificial biofloc of silicate solubilizing bacteria was prepared and maintained to evaluate the biocontrol response of silicate solubilizingbacterial isolates, against *P.oryzae* and the following treatments were tested.

1) Control (No inoculation)
2) SSB-8
3) SSB-11
4) SSB-17
5) Co-inoculation 8+ 11+ 17
6) Natural biofloc of SSB
7) Artificial biofloc of SSB

Bioflocculation assay

One ml aliquot of this culture, silicate solubilizing bacteria, was mixed together in 10 ml bioflocbuffer anduninoculated buffer served as control. The mixtures were vortexed for 10 sec, shaken on a rotary platform shaker for 3 minand left undisturbed at room temperature for 24 hr. All bioflocculation reactions were performed in triplicate.

Estimation of natural bioflocculation of SSB isolates

All the three SSBisolates*viz.,* SSB-8, SSB-11 and SSB-17 were subjected to natural bioflocculation by employing the basal medium of Neyra and Van Berkum (1977), supplemented with Pectic acid and KNO_3, as sole carbon and nitrogen source, at 0.1% and 0.005% concentration, respectively.

Estimation of artificial bioflocculation of SSB isolates

The inoculants of all the three SSB isolates *viz.,*SSB-8, SSB-11 and SSB-17 were prepared as described in chapter 3.19.2. Hundred ml volumes of SSB broth (Bunt and Rovira, 1955) supplemented with 0.05 per cent yeast extract (w/v) were dispensed in 250 ml Erlenmeyer flasks.

One ml culture of each SSB isolate was added separately and aseptically to the medium and incubated under static condition at 30±2°C for 24 hr. After the incubation period, 10 ml of 5% each plant seed extract*viz.,Moringaoleifera, Strychnospotatorum, Allium cepa, Sappindu semaginatus* and *Aestracantha longifolia* was added individually and aseptically into the medium, shaken on a rotary platform shaker for 3 min and left undisturbed at room temperature for 1 hr. The flocculation per cent and floc dry weight of 77SSB isolates were estimated.

Comparison of Natural and Artificial Bioflocculation of SSB Cells with respect to EPS and PHB production at various time intervals

All the three SSBisolates *viz.,* SSB-8, SSB-11 and SSB-17 were subjected to natural bioflocculation by employing the basal medium of Neyra and Van Berkum (1977), supplemented with Pectic acid and KNO_3, as sole carbon and nitrogen source, at 0.1% and 0.005% concentration, respectively. The same isolates were subjected to artificial bioflocculation by using different plant seed flocculants *viz.,Moringaoleifera, Strychno spotatorum, Allium cepa, Sappindus emaginatus* and *Aestracantha longifolia.*

Response of SSB Exopolysaccharides (EPS) and ISR inducing chemicals on the enhancement of growth and blast disease incidence in rice

The paddy seeds were surface sterilized, allowed to germinate, growth chamber was prepared,the germinated seeds were placed in the growth chamber and incubated**.**

The purified EPS of SSB isolates *viz.,* SSB-8, SSB-11 and SSB-17 wereprepared described byKyunseuk *et al*., 2008 and adjusted to 200 ppm concentration with sterile distilled water. The ISR inducing chemicals *viz.,* salicylic acid, jasmonic acid and Azibenzolar at a level of 0.01 per cent were also prepared separately. After 10 days of incubation, ISR inducing chemicals and purified EPS preparation of SSB isolates were in filtered into the cotyledons of rice plants, separately while the controlplant was infiltrated with sterile water. Three replicates were maintained for each treatment. Five days after infiltration, the rice plants from each treatment were challenge inoculated with spore suspension of *Pyricularia oryzae*One week after challenge inoculation, the disease incidence was recorded .

RESULTS

Effect of different formulations of silicate solubilizing bacterial cells on the incidence of rice blast disease (P.*oryzae*) in i*nvitro* condition.

The application effect of the different formulations of silicate solubilizing bacterials cells, namely the vegetative cells of silicate solubilizing bacteria and the natural and artificial biofloc of silicate solubilizing bacteria on the incidence ofblast disease (P.*oryzae*) was studied under *in vitro* condition and the results are presented in Table.1.

Table.1. Effect ofthe application different formulations of SSB isolates on blast disease incidence in lowland rice cv. IR-50

Sl. No.	Treatments[a]	Percentage of disease incidence[b]	Statistics[c]
1.	Control (uninoculated)	88.10±1.81	G
2.	*B. mucilaginosus* vegetative cells + SSB-8	42.40±0.83	E
3.	SSB-11	36.80±0.30	D
4.	SSB-17	44.60±0.85	F
5.	Co-inoculation of 8+11+17^{++}	21.10±0.24	C
6.	Biofloc (Natural)$^{+++}$	17.11±0.45	A
7.	Biofloc (Artificial)$^{+++}$	20.60±0.56	B

[a] At 1x10^{7}cfu ml^{-1}

[b]Values are mean of three replications ± SD.

[c]Values followed by different letters are significantly differed at 5% level according to student't' test.

Among the different formulations of silicon solubilizing bacterials cells, the natural and artificial biofloc of silicate solubilizing bacteria reduced the incidence of blast disease to a higher level when compared to the vegetative cell forms of the same. Between the two vegetative cell forms silicate solubilizing bacterial vegetative cells were found to record the high incidence ofblast disease (42.40+0.83, 36.80+0.30 and 44.60+0.85) when compared to control. Between the natural and artificial biofloc of coinoculation of silicate solubilizing bacterial cells, the natural biofloc of silicate solubilizing bacterial cells + rice straw was found to reduce the incidence of blast disease of rice to a higherlevel than the artificial biofloc of the same. The study clearly indicated the efficiency of silicate solubilizing bacterial natural biofloc on decreasing the incidence of blast disease when compared to other formulation of the same.

Response of silicate solubilizing bacterial exopolysaccharides(EPS) and ISR inducing chemicals on the enhancement of growth andincidence of blast disease (*P.oryzae*) in rice.

A comparative study on the role of purified EPS of silicate solubilizing bacteria isolates viz., SSB-8, SSB-11 and SSB-17 and ISR inducing chemicals namely salicylic acid, jasmonic acid and azibenzolar on the growth and blastdisease incidence in rice was studied under *in vitro* condition and the results presented in Table.2.

Table.2. Response of rhizo bacterial exopolys accharides (EPS) and ISR inducing chemicals on the enhancement of growth and blast incidence (*Pyriculariaoryzae*) in lowland rice under pot culture condition

Sl. No.	Treatment	Plant height*** (cm)	Disease incidence (%)[ab]
1.	Control	16.90±1.44	76.70±1.69
	ISR inducing chemical**		
2.	Salicylic acid	17.18±0.87	21.99±1.37
3.	Jasmonic acid	16.84±0.60	21.74±0.94
4.	Azibenzolar	16.84±0.39	22.22±1.12
5.	EPS of SSB*	22.08±0.72	19.30±0.98
	(co-inoculation)		
6.	EPS of Biofloc (natural)*	25.61±0.36	18.92±0.57

* EPS collected from minimal medium of Neyra and VanBerkum (1977) supplemented with 0.1% pectic acid and 0.005% KNO_3 after 48 hr of incubation. Purified EPS was prepared.

** at 0.01 per cent

*** 20* DAS

[a]Disease incidence estimated 7 days after challenge inoculation with *Pyriculariaoryzae*

[b]Values are mean of three replications ± SD.

The EPS application of each silicate solubilizing bacterial isolate was found to enhance the plant height of rice and reduced the disease incidence in rice. Interestingly, the application of EPS collected from the silicate solubilizing bacterial isolates augmented the height of rice plant and reduced the disease incidence to a higher level when compared to the application of ISR including chemicals. Eventhough, the application of ISR inducing chemicals was also found to reduce blast disease incidence as in the case of purified EPS application of silicate solubilizing bacterial isolates but did not augment the growth of the rice plant. The results of the present study clearly revealed the absence of phyto stimulatory activities of these chemicals. The results of the present study alsosuggested the dual effect of silicate solubilizing bacterial EPS 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.

DISCUSSIONS

Silicon normally deposited mostly on graminaceousplants and increased the cell strength and rigidity. However Si might play an active role in enhancing host resistance to plant disease by stimulating defense mechanism and leads to reduction in disease incidence in many crop plants. Silicon is an important constituent of rice, which gets accumulated in almost every part of the plant. As silica is not applied as any form of fertilizer, plant has to depend on soil for its total requirement. Although it appears that soil contains enough of this element but the same exists in an unavailable from for absorption by plant roots. Hence the bio dissolution of silicon is the need of the hour for making the bioavailability of the same (Kutuzova, 1969; Sheila Partrick and AjHoldin, 2008).The positive role of *Bacillus* as silicate solubilizing bacterium, in rice ecosystem has been reported by many workers(Muralikannan and Anthoniraj, 1996).

Bacillus mucilaginosus, plays a significant role on enhancing the silicon nutrition. Plant growth promotion and biocontrol of *Pyricularia oryzae* in rice crop (Datnoff *et al*., 1991). Silicate solubilizing organisms secrete plant growth promoting substances such as IAA and GA in the rhizosphere and promote the growth and yield of rice. Hence the development of this organism, as agricultural bio-inoculant needs to be exploited in detail for the minimization of growth and yield in rice (Lain Bin *et al*., 2002). Even though very few reports suggested the positive role of *B.mucillagenosus* inoculation in rice crop, the role of EPS rich *Bacillus*bioflox application on the minimization of growth and yield of rice crop has not been studied so far. Moreover the effect of silicon as an elicitor of ISR against P.*oryzae*has been evaluated.

Neyr *et al.,* 1999 reported the positive effect of *Azospirillum* and *Rhizobium*bioflx in augmenting the growth and yield of Faba bean.

Greater plant height of rice due to the inculationof SSB has been reported by many authors(Barbiei *et al*., 1998; Agarwal and Singh, 2000). In the present study it was observed that the application ofSSBbioflx (natural) consisting of different efficient SSB strains viz, SSB-8, SSB-11 and SSB-17 augmented the plant height and ISR mediated biocontrolagainst P. *oryzae*.

CONCLUSION

The present investigation showed improved plant height as well as ISR mediated biocontrol against P. *oryzae* in IR 50 rice crop due to the application of silicate solubilizing bacteria in the form of biofix(natural) and organic siliceous material–rice straw. Hence it is proposed that the EPS rich stress tolerant intergeneic SSB biofloxcontaining efficient silicate solubilizing bacterial isolates as a novel delivery system and new generation of agricultural bio-inoculant along with organic siliceous rice residues supplementationwhich could be used in rice crop under low land condition and the application of the same enhanced the plant growth stimulation and IRS mediated blast disease biocontrol which eventually lead to the maximizing the rice productivity (yield / ha) grown under lowland condition.

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