ISSN:0971-1260

Vol-22-Issue-10-November-2019

Studies On The Effect Of Silicate Solubilizing Bacteria(Ssb) For Management Of Rice Blast *(Pyricularia oryzae)*

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ABSTRACT

Induced Systemic Resistance (ISR) has emerged as a potential tool in crop protection practices and suitable strategy for the reduction in the use of synthetic chemical pesticides and purely based on biological control. Induced systemic resistance can be defined as the phenomenon by which plants exhibit increased level of resistance to broad spectrum of phytopathogens by the prior activation of genetically programmed defence pathways . The colonization of roots with selected microbial strains can also lead to induction of systemic resistance, commonly denoted as '**ISR**'. Induction of systemic resistance by selected strains of microbial has been proved spatially separating the pathogen and microbes in plants. Several bacterial determinants of microbial origin strains *viz.*, lipopolysaccharides (LPS), siderophores (Us, salicylic acid and EPS have been reported to be responsible for the induction of systemic resistance in host plants.The resistance of rice to several diseases, namely, brown spot (*Helminthosporium oryzae* Breda de Haan), stem rot (*Leptosphaeria salvinii* Catt.) and blast disease (*Pyricularia oryzae* Cav.) is stated to increase where the silica content of the plant particularly in the leaves and it has been raised by application of siliceous slags to the soil.A comparative

ISSN:0971-1260

Vol-22-Issue-10-November-2019

study on the role of Purified EPS of silicate solubilizing bacterial isolates *viz*., SSB-8, SSB-11 and SSB-17 and ISR inducing chemicals, namely, salicylic acid, jasmonic acid and azibenzolar on enhance the growth and *Pyricularia oryzae* disease incidence in rice was studied under *in vitro* condition.

Key words: ISR, EPS, *Pyricularia oryzae* , *SSB (Bacillus mucilaginosus)*.

INTRODUCTION

In soils amended with calcium silicate, rice yields has been found to be increased significantly due to reduction in disease severity (Datnoff *et al.,* 1991; Snyder *et al*., 1986). incotton. Silica promotes the photosynthetic activity of P utilisation in rice (Ma and Takahashi, 1990).

Plants growing under natural conditions do not appear to suffer from Si deficiencies. However, Si containing fertilizers are routinely applied to several crops including rice (Pereira *et al.*, 2004) and sugarcane (Savant *et al.*, 1999) to increase crop yield and quality. Increased silicon supply improves the structural integrity of crops and may also to improve plant tolerance to disease, drought and metal toxicities (Epstein, 1999; Richmond and Sussman, 2003; Ma, 2004).

Silicon deposition in the cell walls of root endodermal cells may contribute to the maintenance of an effective apoplastic barrier and there by improve the plant resistance and to disease the drought stresses (Lux *et al.*, 2002; Hattori *et al*., 2005).

Rodrigues *et al.* (2001) investigated the ultrastructural outcome of the Rice - *M grisea* interaction upon Si application. They provided the first cytological evidence that Si-mediated resistance to *M. grisea* in rice correlated with a specific leaf cell reaction that interfered with the development of *M. grisea.* The possibility that this amorphous material contains phenolic compounds appears realistic, considering not only its staining with toluidine blue and its texture and osmophilic properties, but also the occurrence of marked fungal hyphae alterations. Cytochemical labelling of chitin revealed to difference in the pattern of chitin localization over fungal cell walls of either samples from plants amended or not with Si at 96 hr after inoculation with*M. grisea* indicating limited production of chitinases as one

ISSN:0971-1260

Vol-22-Issue-10-November-2019

mechanism of rice defense response to blast. On the other hand, the occurrence of empty fungal hyphae, surrounded or trapped in amorphous material, in samples from plants amended with Si suggested that phenolic-Iike compounds or phytoalexin(s) played a crucial role in rice defense response against infection by *M. grisea.* Therefore, Si could be acting as a modulator to positively amplify rice defense response(s), namely by influencing the synthesis of antifungal compounds after the peg penetration of *M grisea* into the epiderminal cell. Effect of Silicate solubilizing bacterial EPS on the growth and disease resistance on rice plant were studied.

MATERIALS AND METHODS

RESPONSE OF SSB EXOPOLYSACCHARIDES (EPS) AND ISR INDUCING CHEMICALS ON THE ENHANCEMENT OF GROWTH AND BLAST DISEASE INCIDENCE IN RICE

The surface sterilization, germination, preparation of growth chamber, placement ofgerminated rice seeds in growth chamber, Incubation of growth chamber were done. The purified EPS of SSB isolates *viz.,* SSB-8, SSB-11 and SSB-17 was prepared according to Kyunseuk *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.

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 according to Kyungsedk *et al*. (2008). at 0.01 per cent20DASDisease incidence estimated 7 days after challenge inoculation with *Pyricularia oryzae*Values are mean of three replications ± SD.absence of phytostimulatory activities of these chemicals. The results of the present study also suggested 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. For incubation, ISR inducing chemicals and purified EPS preparation of SSB isolates were infiltered into the cotyledons of rice plants, separately whereas the control.

ISSN:0971-1260

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Plant was infiltrated with sterile water. Three replications were maintained for each treatment. Five days after infiltration, the rice plants from each treatment was challenge inoculated with spore suspension of *Pyricularia oryzae*. One week after challenge inoculation, the disease incidence was recorded as per the procedure.

EXPERIMENTAL RESULT

Response of silicate solubilizing bacterial Exopolysaccharides (EPS) and ISRinducing chemicals on the enhancement of growth and blast disease incidence (*Pyriculariaoryzae*) in rice

A comparative study on the role of Purified EPS of silicate solubilizing bacterial isolates *viz*., SSB-8, SSB-11 and SSB-17 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.

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 inducing 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.

Response of rhizobacterial exopolysaccharides (EPS) and ISR inducing chemicals on the enhancement of growth and blast incidence (*Pyricularia oryzae*) 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

ISSN:0971-1260

Vol-22-Issue-10-November-2019

	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* (co-inoculation)	22.08±0.72	19.30±0.98
6.	EPS of Biofloc (natural)*	25.61±0.36	18.92±0.57

DISCUSSION

The application of SSB isolates in the form of biofloc (natural) along with organic siliceous material and challenge inoculation of *Pyricularia oryzae* augmented the growth and yield parameters *viz*., plant height. 1000 grain weight of IR 50 rice and reduced the plant disease incidence to a maximum level followed by SSB alone treatments and provided the indirect evidence that the plant growth stimulation due to silicon nutrition and silicon mediated ISR against blast biocontrol and also the same application of the SSB biofloc formulation organisms increases P, silicate and also chlorophyll content in rice (IR 50).

The present investigations showed improved growth parameters and yield parameters as well as ISR mediated biocontrol against *Pyricularia oryzae* in IR 50 rice crop, by the application of silicate solubilising bacteria in the form of biofloc (natural) and organic siliceous material-rice straw.

Hence, it is proposed that the "EPS rich, stress tolerant Intergeneric SSB bioflocs", containing efficient silicate solubilising bacterial isolates as a novel delivery system and new generation of agricultural bio-inoculant along with organic siliceous rice residues supplementation could be used in rice crop under lowland condition and the application of the same enhanced the survival, aggressive colonization, plant growth stimulation and ISR

ISSN:0971-1260

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mediated blast disease biocontrol which eventually lead to the maximization of rice productivity (yield/ha) grown under lowland condition.

Resistance of rice plants amended with Si in response to *M. grisea* infection, twomutually agreeable hypothesis must be considered. On the other hand, it is possible that in certain areas of heavy Si deposition, delayed fungal infection and colonization provides the rice plant enough time for momilactones synthesis in response to infection by *M. grisea*, to accumulate and to considerable levels and express its fungitoxicity within the zone of the infection site. Fawe *et al.* (2001) proposed that soluble Si present in the lilt cells may mediate some defense responses that are functionally similar systemic acquired resistance, the results of this study, together with the infrastructural observations, strongly suggested that Si plays an active role in resistance of rice to blast rather than simply forming a physical barrier in leaf epidermis to impede fungal penetration.

Rodrigues *et al.* (2004) tested the hypothesis that an alteration in the development of *M. grisea* in leaf tissues of rice plants amended with Si could be associated with an enhanced production of phytoalexins. Analysis of the ethyl ether fraction (FII) obtained from leaf extracts of plants amended with (Si^+) or without (Si^-) and inoculated with *M. grisea* revealed among of the five sub-fractions (SF) collected. The fraction SF 5, which corresponded to compounds eluting after 90 min. in the HPLC chromatograms, displayed antifungal activity against *M. grisea*. The SF 5 from Si^+ treatment showed higher fungitoxicity against *M. grisea* than SF 5 from Si treatment.

Two mechanisms for Si-enhanced resistance to diseases have been proposed. One is that Si acts as physical barrier and is deposited beneath the cuticle to form a cuticle - Si double layer (Fauteux, 2005). This layer can mechanically impede penetration by fungi and thereby disrupt the infection process. Another mechanism proposed recently is that soluble Si acts as a modulator of host resistance to pathogens.

Several studies in monocots (rice and wheat) and dicots (cucumber) have shown that plants supplied with Si can produce phenolics and phytoalexins in response to fungal infection such as those causing rice blast and powdery mildew (Fawe *et al.*,1998;

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Belanger *et al*.,2003; Rodrigues *et al*.,2004; Remus-Borel, 2005).

In rice, differential accumulation of glucanase, peroxidase and PR-I transcripts were associated with limited colonization by the fungus *M. grisea* in epidermal cells of a susceptible rice cultivar supplied with Si (Rodrigues *et al*., 2005). These biochemical responses are only induced by soluble Si, suggesting that soluble Si might play an active role in enhancing host resistance to diseases by stimulating some mechanisms of the defense reaction (Fauteux, 2005). However, the exact nature of the interaction between the soluble Si and the biochemical pathways of the plant that leads to disease resistance and remains unknown although several possible mechanisms have been proposed (Fauteux *et al*., 2005).

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ISSN:0971-1260

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