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Induction of systemic resistance in rice against blast disease by bioagents and chemicals

A. P. Gaikwad and Y. S. Balgude

Agricultural Research Station, Lonavala , Pune 410 401 (MS), India
Email: apgaikwad@rediffmail.com, ars_lonawala@rediffmail.com

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Abstract

Three bioagents *viz., Pseudomonas fluorescens, Trichoderma harzianum* and *Bacillus subtilis* as well as the chemicals like Jasmonic acid (JA), Salicylic acid (SA), Benzothiadiazole (BTH), Abscisic acid (ABA), Potassium hydrogen phosphate, Oxalic acid, Copper chloride with fungicide carbendazim were tested for inducing systemic acquired resistance (SAR) against *Pyricularia grisea* (*Magnaporthe grisea)* in rice. The talc-based formulation of bioagents and chemical application of chemicals as a suspension through seed soaking, soil and foliar application in combination (seed + soil + foliar) were tried. Among the SAR inducers, the treatment combination of *P. fluorescens* + *T. harzianum*+ *B. subtilis* (each @ 10^8 cfu ml^{-1}) was found to be most effective in management of leaf blast of paddy and promoted plant growth under glasshouse conditions. This was followed by *P. fluorescens* (10^8cfu ml^{-1}) alone, jasmonic acid (0.1 mM) and salicylic acid (0.1 mM). The efficacy of *P. fluorescens* + *T. harzianum* + *B. subtilis* was comparable to that of the fungicide carbendazim. However, carbendazim (0.10%) was superior over all SAR agents. The extent of increase varied between treatments. The combined application of bioagents through all of the three (seed, soil and foliar) methods increased the SAR in rice plants. The results indicated that the combination of biological agents showed a better response to fight against rice blast pathogen *P. grisea* than the treatment alone. Thus the present study shows that the induction of defense related enzymes enhance resistance against invasion of *P. grisea* in rice.

Key words: Systemic resistance, rice blast, bio agents, chemicals

Introduction

It's a known phenomenon that the physical and biotic factors modulate the metabolic pathways in plants. They result into triggering of trigger defense responses in plants against detrimental factors i.e. physical stresses or pathogens. This phenomenon is collectively called as resistance induction and the agent responsible for this phenomenon is termed as resistance inducer. Resistance has been induced either by using biotic or abiotic stimuli. Variation of abiotic factors such as temperature, radiations and chemicals may induce a number of defense compounds in plants. Biotic factors namely bacteria, fungi and nematodes have also potential to alter biochemical profile of plants. All these factors directly alter the rate of transcription and translation processes in plant cells. (Shafique, 2011).

Systemic acquired resistance (SAR) is the phenomenon whereby a plant's own defense mechanisms are induced by prior treatment with either a biological or chemical agent. The concept of SAR has been widely recognized and studied for the past 100 years in relation to increasing resistance to fungal, bacterial, and viral pathogens of economically important crop plants (Glynn, 2001). Plants have evolved a battery of sophisticated defense mechanisms to defend themselves against microbial pathogens. Experiments with crop plants have shown that SAR can lead to long-lasting, broad spectrum disease control and can be used preventively to bolster general plant health. Ample evidence suggests that SAR is based on multiple natural defense mechanisms, and this makes it less likely that a pathogen can readily develop resistance to this control measure (Kessmann *et al*., 1994). The availability of this long-lasting, broad-spectrum and potentially stable solution to disease control may have a positive impact on rice blast management.

The resistance inducing chemicals such as oxalic acid, salicylic acid, ascorbic acid and salt [KCl, K_2HPO_4 and Na_2HPO_4], Indole Acetic Acid [IAA], Indole Butyric Acid [IBA] (Ragab *et al*., 2010); abscisic acid [ABA] (David De Vleesschauwer, 2010); β-Aminobutyric acid [BABA] (Walters *et al*. 2013) and jasmonic acid [JA] (Wasternack and Hause, 2013 and Shoko, *et al*., 2012) as well as bioagents *viz.,P. fluorescens* (Krishnamurthi and Gannamanickam, 1997 and Anita and Samiyappan, 2012); *Bacillus* sp. and *Trichoderama* spp. (Vasudevan *et al*., 2002) were tested previously by several workers for inducing resistance against different diseases in various crops.

Rice (*Oryza sativa* L.) is the most important staple food grain for the people living in the rural and urban areas of humid and sub-humid Asia. The productivity of rice is less (1.8 t/ha) in Maharashtra as compared to India (2.41 t/ha) [Anonymous, 2014]. Among the several diseases infecting rice, blast disease caused by *Pyricularia grisea* (*Magnaporthe grisea) is important in rice* in Maharashtra which causes about 10-80 per cent yield loss. Repeated use of the chemical pesticides is causing severe concern from the health and environmental point of view. In view of these, the attempts were made to manage the disease by inducing the systemic acquired resistance against blast disease in rice by use of different non hazardous chemicals and bioagents.

Materials and methods

A pot culture experiment was conducted in CRD with three replications at Agricultural Research Station, Lonavala during 2014. The chemical inducers *viz.*,jasmonic acid (JA), salicylic acid (SA), benzothiadiazole (BTH), abscisic acid (ABA), potassium hydrogen phosphate (K_2HPO_4), oxalic acid, copper (II) chloride ($CuCl_2$) and bioagents like *P. fluorescens, T. harzianum* and *B. subtilis* were tried against blast of paddy at the concentration as indicated in Table 1. The pathogen culture *P. grisea* isolated on PDA and multiplied on oat meal broth was used for the studies. Similarly, the talcum powder based microbial cultures of bioagents were obtained from the BNF scheme, College of Agriculture, Shivajinagar, Pune.

Induced resistance assays were performed as described by De Vleesschauwer *et al.* (2006). The plants were grown under glass house conditions at 25 ± 3°C temperature and 12-h photo period by sowing 25 seeds in each pot containing sterilized soil + compost (4:1) that had been autoclaved twice on alternate days for 21 min. Rice seeds of susceptible cultivar, EK-70, first were surface sterilized with 1% sodium hypochlorite for 2 min, rinsed three times with sterile, demineralized water and soaked in the suspensions of different chemical and biological inducers for 24 hrs. The drenching of inducers was done ten days after sowing @ 25 ml/pot having diameter of 15 cm. Thereafter, two sprays of inducers were taken with the help of atomizer at 15 and 25 days after sowing until runoff. As a positive control, plants were sprayed with carbendazim (0.1 %). At 28 days after sowing, i.e. three days after second spray of inducers, the rice plants were challenge inoculated with *P. grisea* by spraying the spore suspension containing virulent conidia (3 x10^4 spores/ ml) with atomizer and the control plants were sprayed with distilled water. High humidity was created by preparing muslin cloth and polythene paper canopy and by sprinkling the water frequently in the pot culture 24 and 48 hrs prior to and after inoculation. The observations on leaf blast diseases were recorded 40 days after sowing, i.e. 12 days after challenge inoculation, by following 0 - 9 SES scale (Anonymous, 2002) and then converting into per cent disease intensity by using the formula.

$$\text{Per cent disease intensity} = \frac{\text{Sum of the scores X 100}}{\text{Numbers of observations X highest rating i.e. 9}}$$

The observations for growth parameters like plant height and number of tillers were recorded 60 days after sowing.

Results and Discussion

Leaf blast

Data presented in Table 1 indicate that the treatment differences due to SAR inducing agents and fungicide were statistically significant. Significantly lower disease intensity (30.37 per cent) was noticed in the treatment *P. fluorescens + T. harzianum + B. subtilis* (each @ 10^8 cfu ml^{-1}) with disease reduction of 53.31 per cent. However, it was at par with *P. fluorescens* (10^8cfu ml^{-1}), jasmonic acid (0.1 mM) and salicylic acid (0.1 mM), wherein the leaf blast intensities were 33.78, 34.96 and 36.30 per cent with disease reduction of 48.07, 46.24 and 44.19 per cent, respectively. It was followed by copper chloride (0.05 mM), oxalic acid (0.05 mM), abscisic acid (0.1 mM), *B.subtilis* and *T. harzianum* (Both @ 10^8cfu ml^{-1}), which were at par with each other and had 40.30, 42.81, 43.11, 43.70 & 44.00 per cent intensity with 38.04, 34.17, 33.72, 32.80 & 32.35 per cent reduction of leaf blast, respectively.

Table 1: Influence of bioagents, growth promoters and fungicides on leaf blast and plant growth parameters in paddy

Tr. No.	Treatments	Conc. (% / mM)	Per cent leaf blast			Plant growth parameters	
			Incidence	Intensity	Control	Height (cm)	No. of tillers
1	Jasmonic acid (JA)	@0.1 mM	74.67 **59.79**	34.96 **36.24**	46.24	24.63	4.33
2	Salicylic acid (SA)	0.1 mM	76.00 **60.72**	36.30 **37.02**	44.19	23.67	4.00
3	Benzothiadiazole (BTH)	0.5mM	Heavy phytotoxicity				
4	Abscisic acid (ABA)	0.1 mM	86.67 **69.44**	43.70 **41.36**	32.80	19.33	3.00

5	Potassium hydrogen phosphate (K2HPO4)	20 mM	88.00 **69.91**	45.93 **42.66**	29.39	18.03	2.33
6	Oxalic acid	0.5 mM	84.00 **66.53**	42.81 **40.87**	34.17	21.77	3.33
7	Copper(II) chloride (CuCl2)	0.05 mM	80.00 **63.51**	40.30 **39.38**	38.04	22.40	3.67
8	*Pseudomonas fluorescens* (*P.s.*)	10^8cfu ml^{-1}	73.33 **59.08**	33.78 **35.52**	48.07	25.73	4.67
9	*Trichodermaharzianum* (*T.h.*)	10^8cfu ml^{-1}	86.67 **68.91**	44.00 **41.55**	32.35	19.00	2.67
10	*Bacillus subtilis* (*B. s.*)	10^8cfu ml^{-1}	85.33 **67.81**	43.11 **41.02**	33.72	20.30	3.00
11	*P. f.* + *T. h.* + *B. s.*	10^8 cfu ml^{-1} each	70.67 **57.28**	30.37 **33.41**	53.31	27.20	5.00
12	Carbendazim	0.01	40.00 **39.22**	13.19 **21.25**	79.73	29.30	5.33
13	Control	-	100.00 **85.95**	65.04 **53.76**	-	16.23	2.33
	S.E.$\pm$		2.49	1.32		1.70	0.71
	C.D. (0.5%)		7.31	3.87		4.98	2.07
	C.V.(%)		6.75	5.09		13.2	33.63

Note: Figures in bold faces are arc sine values

Earlier, several studies have demonstrated the induction of resistance in rice against various diseases following treatment with antagonistic rhizobacteria *P. fluorescens* and resistance inducing chemical salicylic acid. Ragavan (2003) and Shyamala (2012) also reported that the inoculation of *P. fluorescens* and salicylic acid improved resistance in rice against *P. grisea,* which is in agreement with present findings. De Vleesschauwer (2008) demonstrated that treatment of rice plant with *P. fluorescens* induced resistance against *P. grisea*. Vidyasekaran (1997) reported that pretreatment of rice leaves with *P. fluorescens* as a seed and foliar spray effectively controlled the disease. The results of the present study confirmed the systemic nature of resistance against the pathogen, which is induced through exogenous application of SA. Similar results were recorded on the effect of SA on induction of systemic resistance by Mills and Wood (1984) in cucumber against *Colletotrichum* sp.

The treatment with fungicide carbendazim (0.10%) was significantly superior over all SAR agents wherein the incidence, intensity and blast reduction were 40.00, 13.19 and 79.73 per cent, respectively. The untreated control had significantly highest incidence of 100.0 per cent and severity of 65.04 per cent of the disease.

Plant growth parameters

The data regarding plant growth parameters indicated that the treatment differences due to plant height and number of tillers were statistically significant. Significantly highest plant height and no. of tillers were recorded in the treatment combination of *P. fluorescens* + *T. harzianum*+ *B. subtilis* (each @ 10^8 cfu ml^{-1}), wherein these parameters were 27.20 cm and 5.0, respectively. Whereas, it was at par with *P. fluorescens* (10^8cfu ml^{-1}), jasmonic acid (0.1 mM), salicylic acid (0.1 mM) and copper chloride (0.05nM), where the plant height was 25.73, 24.63, 23.67 and 22.40 cm, while No. of tillers were 4.67, 4.33, 4.0 and 3.67, respectively. These results are in conformity with the reports of Ragavan (2003) and Shyamala (2012) who also noticed the reduction of blast and improved plant growth in rice due to inoculation of *P. fluorescens* and salicylic acid.

Conclusion

Among the SAR inducers, the combination of *Pseudomonas fluorescens* + *Trichoderma harzianum*+ *Bacillus subtilis* (10^8 cfu ml^{-1} each) was found to be most effective in management of leaf blast and improving plant growth of paddy followed by *P. fluorescens* (10^8cfu ml^{-1}), jasmonic acid (0.1 mM) and salicylic acid (0.1 mM). However, the fungicide carbendazim (0.10%) was superior over all SAR agents.

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