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SHORT COMMUNICATION

PHYSIOLOGICAL EVALUATION OF *TRICHODERMA HARZIANUM* AGAINST SHEATH BLIGHT IN RICE

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***Trichoderma harzianum* (LS: TH38) induced systemic resistance in rice (*Oryza sativa*) by enhancing various components involved in pathogen suppression. Different physiological processes that are related with growth promotion were positively induced by *T. harzianum* even under infection by sheath blight. Thus reduction in disease severity and nullifying its negative impact on the plant growth implicates the importance of *T. harzianum* as a biocontrol agent. However, comparative study with systemic fungicide bavistin showed that the efficacy level of *T. harzianum* against sheath blight disease needed further improvement in order to replace chemical fungicides.**

***Key words*: Bavistin, physiological processes, sheath blight, systemic resistance, *Trichoderma harzianum*.**

Sheath blight of rice (*Oryza sativa* L.) is caused by *Rhizoctonia solani* Kuhn., a common soil borne pathogen, is a serious disease of rice in all rice growing countries and responsible for 6 to 10% of annual yield loss (Mew *et al*. 2004). Although use of fungicides for disease suppression gives promising results, it is environmentally undesirable and economically unsustainable. Recent studies have demonstrated that, *Trichoderma harzianum* is an opportunistic, avirulent plant symbiont and shows unique biocontrol activity (Cardona and Rodriguez 2006). It also induces many defense components involved in systemic resistance and simultaneously influence the growth physiology by enhancing nitrogen assimilation and nutrient uptake (Harman *et al*. 2004). Although many reports are available for the overall impact of biocontrol agents and commercial fungicides on disease suppression and yield, a systemic and comparative approach is needed to explore their effect on key physiological processes that contribute to growth and resistance. In view of this, present investigation was carried out for the comparative evaluation of resistance inducer *T. harzianum* and commercial systemic fungicide bavistin (carbendazim) for their ability to suppress sheath blight disease as well as their impact on the physiology of rice under green house conditions.

A pot experiment was designed in sterilized soil under green house condition. Seeds of rice variety (Vasumati) were bioprimed with solid formulation powder of *T. harzianum* LS: TH38 (5gm formulation/kg seed) in a moist chamber with relative humidity of 80-85per cent for overnight at 30^0C. Bavistin treatment was given for eight hours by dipping rice seeds in 1mM solution and then thoroughly washing with distilled water. Two subsequent sprays of *T. harzianum* (5% w/v) and 1mM bavistin solution were performed at 10, 30 and 60 days after transplanting. Plants were separately inoculated

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with the sheath blight pathogen, *Rhizoctonia solani* AG 1A, at active tillering stages following the method given by Singh *et al*. (2002). Plants infected without treatment (IWT) were taken as negative control while untreated plants without infection were taken as positive control for comparison.

Concentration of chlorophyll *a*, chlorophyll *b*, total chlorophyll (chl *a* + chl *b*) and carotenoid content (mg g^{-1} fw) from uppermost leaves were analyzed spectrophotometrically following the method of Chaum *et al*. (2004). Photosynthesis (µ106/4mol CO_2 m^{-2} s^{-1}), was determined in full sunlight (PAR~ 1400 to 1600) with a portable CO_2 gas analyzer (CID Inc. USA) on top most intact leaves selected randomly. A handy plant efficiency analyzer (Handy PEA, Hansatech, UK) was used to monitor (Fv/Fm). H_2O_2 content of infected leaf sheaths was measured by the method of Alexieva (2001). *In vivo* nitrate reductase activity (amount of nitrite produced g^{-1} fw hr^{-1}) was assayed through Hagman *et al*. (1971). Peroxidase activity (ΔE g^{-1} fw min^{-1}) was determined at 30^0C by a direct spectrophotometric method of Hammerschmidt and Kuc (1982). Per cent infected tillers / hill, per cent infected panicles / hill and relative lesion height (RLH) were taken as parameters to assess disease severity. RLH was calculated according to formula given by Vidhyasekaran *et al*. (1997).

The experiment was conducted in a completely randomized design with three replications. The data was analyzed statistically for calculating standard error of mean (SEM) and CD was taken at 5% for the comparison.

Chlorophyll b significantly reduced under infection with maximum reduction of 78%. However, total chlorophyll and carotenoid content were reduced to a lesser extent in plants with sheath blight infection (Table 1). It has been reported earlier that chlorophyll b is more sensitive than chlorophyll a due to its structural property, localization and conversion to chlorophyll a during stress (Fang *et al*. 1998). This resulted higher chlorophyll a/b ratio under sheath blight infection. Chlorophyll a/b ratio increased up to 2.68 fold under infection with minimum of (2.44) in *T. harzianum* treated plants (Table1). Effect on photosynthetic rate was found to be low and directly correlated with the loss of total chlorophyll due to infection. Chlorophyll fluorescence (Fv/Fm ratio) decreased significantly (53.48 %) in infected plants (Table 1). The possible reason for this may be the dependence of the processes on ion fluxes and

Table 1. Effect of seed treatment with *T. harzianum* and bavistin followed by inoculation with sheath blight pathogen *Rhizoctonia solani* on physio-biochemical properties of rice.

Effects / Treatments	Chl a (mg/g fw)	Chl b (mg/g fw)	Total chloro-phyll (mg/g fw)	Total carot-enoid (mg/g fw)	Chlorophyll a/b rato	Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)	Chlorophyll fluorescence (Fv/Fm ratio)	H_2O_2 content (µmol g^{-1} fw)	NR activity (µmol NO_2^- produced $µg^{-1}$ protein hr^{-1})	Peroxidase activity (ΔE_{420} min^{-1} g^{-1} fw)
T. harzianum	2.02	0.638	2.65	0.123	2.443	17.58	0.40	2.01	5.259	1.129
Bavistin	2.00	0.402	2.40	0.103	2.974	16.00	0.35	1.14	3.665	0.751
IWT*	1.57	0.154	1.72	0.087	5.997	13.05	0.20	1.57	2.563	0.681
Control	1.99	0.724	2.71	0.121	2.237	17.58	0.43	0.70	4.001	0.393
S.Em.±	0.014	0.023	0.022	0.002	0.54	0.134	0.003	0.059	0.038	0.028
CD at 5%	0.058	0.066	0.065	0.008	1.57	0.390	0.011	0.171	0.110	0.081

* Infection without treatment

Data taken are mean value of three replications

photosystem II (PSII), which are always found to be prone to any type of cellular changes taking place under stress conditions (Yun *et al*. 2000). Besides sensitivity of these parameters to sheath blight infection, *T. harzianum* and bavistin treated plants showed significantly lesser effect of infection on chlorophyll a/b ratio, photosynthetic rate and Fv/Fm ratio. However, *T. harzianum* treated plants were significantly better as compared to bavistin treated plants.

H_2O_2 content increased in infected plants (2.24 fold) as compared to control. H_2O_2 accumulation showed a negative correlation with disease severity in *T. harzianum* and bavistin treated plants with 1.6 to 2.87 fold higher H_2O_2 as compared to positive control (Table 1). Systemic resistance induced by *T. harzianum* depends on the signaling molecules as H_2O_2 which has a crucial role in enhancing the level of resistance (Durrant and Dong, 2004). *R. solani* infection caused significant loss (35.94%) in NR activity which was directly related to disease severity. It was found that rice plants treated with *T. harzianum* showed maximum level (5.25 µmol NO_2^- produced g^{-1} fw h^{-1}) of NR activity which was 43.49 per cent higher than bavistin treated plants and 31.44 per cent greater than positive control (Table 1). Peroxidase is well documented enzyme for its role in various types of stresses, particularly under infection (Vasquez *et al*. 2004). Peroxidase activity was increased 73.28 per cent as compared to positive control under sheath blight infection. However, the level of peroxidase activity was significantly higher in *T. harzianum* treated plants with 2.8 fold high as compared to the positive control and showed a negative correlation with disease severity (Table 1).

Sheath blight infection significantly affected untreated plants with maximum relative lesion height (85.61%) and 100 per cent infection in tillers and panicles, respectively. However, this effect was considerably minimized in both *T. harzianum* and bavistin treated plants. The efficacy level of bavistin to minimize disease severity was significantly higher in comparison to *T. harzianum*, as bavistin treated plants showed lowest level of disease severity with RLH (26.04%), 35.15 per cent infected tillers and 27.4 per cent infected panicles, respectively (Table 2).

In conclusion, *T. harzianum* significantly enhanced the growth physiology of rice under green house conditions and simultaneously reduced the sheath blight severity to a significant level. However, efficacy level of *T. harzianum* was comparatively less than bavistin for disease suppression. Different components related to systemic resistance were considerably high in *T. harzianum* treated plants which implicates the importance of this bioagent for producing pesticide free rice in eco-friendly environment. Approaches like mixture of different biological agents and combination of chemical elicitors with biocontrol agents might produce promising results to achieve the better efficacy level to replace the commercial fungicides.

Table 2. Disease severity evaluation in rice (Vasumati) plants treated with *T. harzianum* and bavistin followed by inoculation with sheath blight pathogen *Rhizoctonia solani* AG 1A.

Treatments	Plant height (cm)	Highest point where lesion was seen (cm)	Relative lesion height (%)	Infected tillers (%)	Infected panicles (%)
T. harzianum	118.33	56.00	47.45	50.18	45.72
Bavistine	100.00	26.00	26.04	35.15	27.40
IWT*	71.00	60.66	85.61	100.00	100.00
Control	102.00	0.00	0.00	0.000	0.00
S.Em.±	3.9409	2.1562	2.380	3.949	5.375
CD at 5%	11.7086	6.6521	7.136	11.838	16.114

* Infection without treatment

Data taken are mean value of three replications.

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