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Bacillus-mediated Cross-protection Against Iron Toxicity and Brown Spot Disease in Lowland Rice

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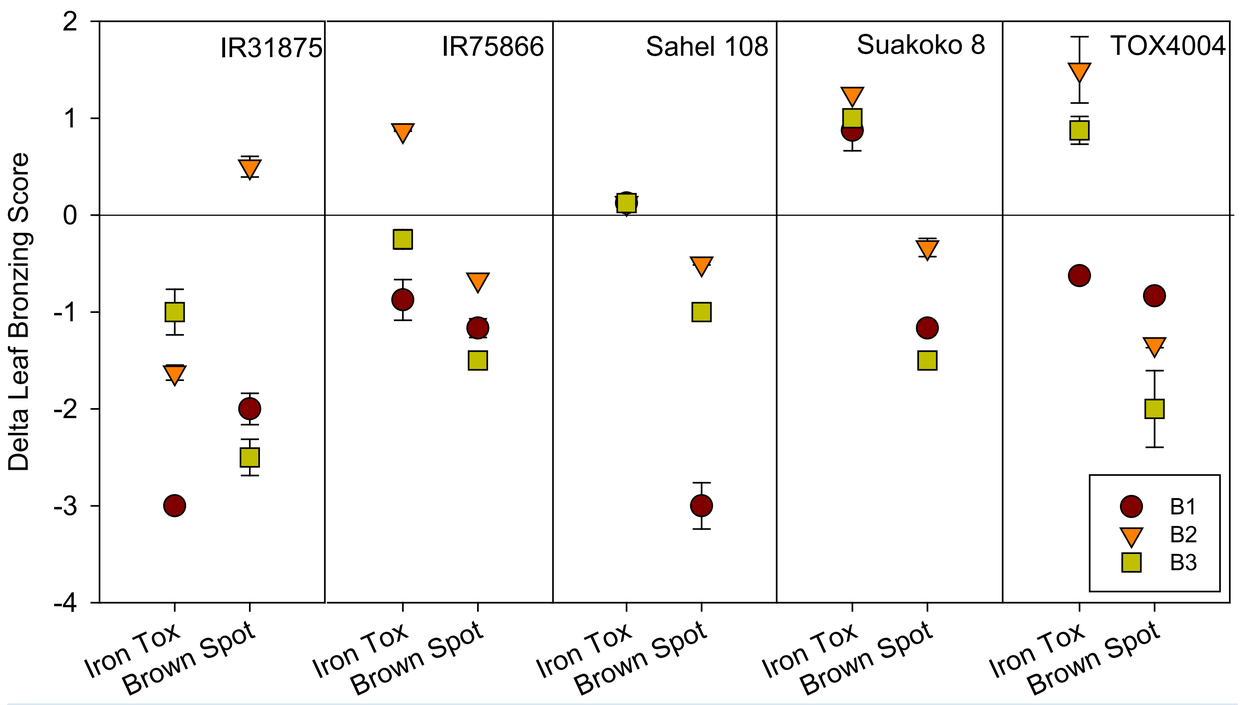
Introduction

Plant fitness can be positively influenced by microorganisms. Microbial strains providing cross-protection against multiple stressors could become a valuable tool in sustainable agriculture.
Iron toxicity and brown spot disease incited by *Bipolaris oryzae* constitute great constraints to lowland rice *(Oryza sativa)* production. We evaluated the effect of three Bacillus isolates on the tolerance of different lowland rice cultivars against iron toxicity and brown spot disease by leaf symptom scoring.

Conclusions

- *B. pumilus* positively affects the resistance against *Bipolaris oryzae* in all 5 cultivars tested.
- The effect on the tolerance to iron toxicity depends on the cultivar x Bacillus strain combination
- Shoot iron distribution can be affected by Bacillus inoculation
- Iron accumulation possibly plays a role in the defense response against *B. oryzae*

Preliminary Results and Discussion

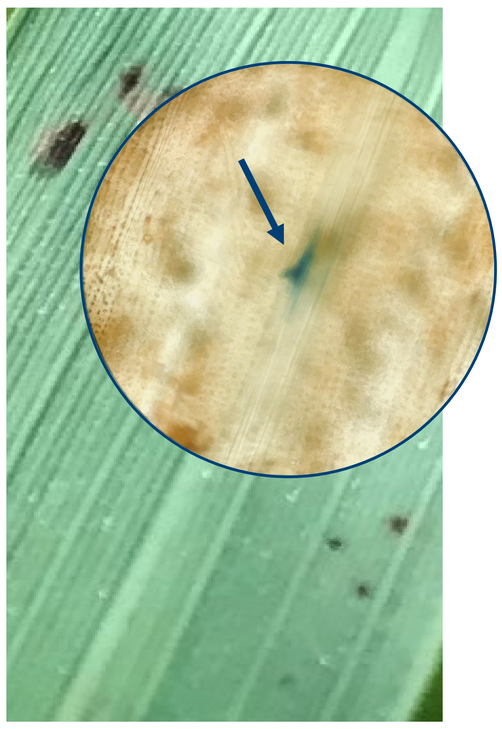


Total shoot iron content of iron-stressed IR31875, Sahel 108, and Suakoko 8 was affected by Bacillus treatment. However, lower shoot iron content did not always result in lower leaf bronzing scores. Closer analysis showed iron content to be differently affected in different organs. (Asterisks indicate significant differences to the non-inoculated control within each organ of the respective cultivar)

Can the effect of Bacillus inoculation on leaf symptom expression be due to altered iron distribution in the shoot?

Perl's staining of leaf blades of rice plants inoculated with *B. oryzae* showed accumulation of Fe III at the entry site of the fungus into the plant cell (blue arrow).

Is iron involved in the defense response of rice against infection with Bipolaris oryzae, possibly as part of a ferroptotic cell death?



Leaf symptom scores of the different lowland rice cultivars were differently affected by Bacillus treatment under iron toxicity and *B. oryzae* infection.
B. pumilus isolates (B1, B3) positively influenced resistance against *B. oryzae* in all cultivars, while tolerance to iron toxicity was only improved in IR31785.

Are the mechanisms underlying the bacterial effects on tolerance to iron toxicity and brown spot disease overlapping?

		Total Iron Content [mg]			
		Bacteria Treatment			
Cultivar	Organ	No Bac	*B1*	*B2*	*B3*
IR31875	Leaf Blade	0.60 ±0.06	0.87 ±0.25	0.77 ±0.08	0.73 ±0.15
	Sheath	0.47 ±0.08	0.63 ±0.06	0.73 ±0.07*	0.50 ±0.05
	Dead Tissue	3.08 ±0.46	1.83 ±0.36 *	1.62 ±0.26 *	1.45 ±0.34 **
	Total Shoot[t]	4.37 ±0.55	3.50 ±0.65	3.30 ±0.39	2.79 ±0.51 *
Sahel 108	Leaf Blade	0.62 ±0.10	0.59 ±0.04	0.50 ±0.08	0.58 ±0.09
	Sheath	0.75 ±0.07	0.71 ±0.07	0.57 ±0.05*	0.62 ±0.09
	Dead Tissue	1.45 ±0.26	2.12 ±0.34	0.92 ±0.09	1.04 ±0.15
	Total Shoot[t]	2.97 ±0.37	3.53 ±0.37	2.08 ±0.16 *	2.37 ±0.29
Suakoko 8	Leaf Blade	0.42 ±0.03	0.71 ±0.1*	0.54 ±0.07	0.51 ±0.03*
	Sheath	0.52 ±0.05	0.75 ±0.06**	0.50 ±0.05	0.40 ±0.08
	Dead Tissue	1.57 ±0.16	2.30 ±0.28 *	1.37 ±0.18	1.38 ±0.23
	Total Shoot[t]	2.65 ±0.19	3.98 ±0.4 **	2.53 ±0.23	2.37 ±0.27

Notes on Materials and Methods

Plants were grown in original Yoshida solution under greenhouse conditions with 12h light/dark period for three weeks before they were inoculated with cell suspensions (10^7 CFU/ml) of three different Bacillus isolates (B1= *B.pumilus* D7.4, B2= *B.megaterium*, B3= *B. pumilus* Ni9MO12 rif.res.). Nutrient solution containing the bacteria was removed after 7 days, prior to iron treatment or *B. oryzae* infection. Iron was applied in the form of Fe (II) ($FeSO_4 \cdot 7H_2O$) to a concentration of 1,000 ppm Fe (II) for 7 days. Hypoxic conditions to prevent iron oxidation in the root zone were induced by N_2 gas diffusion for 15 minutes every two hours and O_2 content monitored. For *B. oryzae* inoculation plants were sprayed with a suspension of spores (5 x 10^4 spores/ml) and transferred into a humid chamber for 2 days.
Leaf bronzing was assessed visually on fully expanded leaves for the entire plant (Asch et al. 2005) 8 days after treatment.
Iron content was measured photometrically in single organs (Hartmann and Asch, 2018). Perl's staining was performed on the blade of the second youngest fully expanded leaf according to Roschzttardtz et al. 2009.