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Alterations to the Physico-Chemical Properties of Rice Grain upon Infection by False Smut Disease and Challenge Inoculation by the Native Bioagents

V. Sowmya [a], P. Maruthi [b], G. Uma Devi [a], J. Aravind Kumar [b], P. Rajanikant [a] and C. Kannan [b*]

[a] Department of Plant Pathology, PJTSAU, Hyderabad, Telanagana-500030, India.
[b] ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telanagana-500030, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

False smut caused by the flower-infecting fungus, *Ustilaginoidea virens* has become an important disease of rice seriously hampering quality and quantity of rice worldwide. Presently, rice false smut (RFS) was generally controlled by synthetic fungicides, which affect grain quality and human health upon consumption. An experiment was conducted to evaluate the effectiveness of native Biological Control Agents (BCAs) in managing false smut disease. The study included both *in vitro* and *in vivo* conditions to assess the ability of four native bioagents (*Trichoderma asperellum* strain TAIK 1, *Bacillus cabrialesii* strain BIK3, *Pseudomonas putida* strain PIK1, and *Pseudomonas otitidis* strain POPS1) in controlling the disease and also increasing the physicochemical properties of grain. The *in vitro* and *in vivo* evaluation revealed maximum inhibition of *U. virens* by consortia of BCAs by 67.54% over the control plants. Besides disease suppression, BCAs were found to be beneficial to

**Corresponding author;*
E-mail: vanamasowmya100@gmail.com;

rice as evidenced by the increase in the hulling, milling and head rice recovery, cooking quality, and nutritional qualities like total protein and amylose content. BCAs will be a management strategy for false smut disease.

Keywords: Trichoderma; bacillus; pseudomonas; grain quality; false smut; ustilaginoides virens; rice.

1. INTRODUCTION

Rice is crucial for global food and nutritional security, providing essential nutrition and energy for around half of the world's population [1]. However, rice production faces significant challenges, including the presence of insect pests and diseases that affect specific regions. One such disease is Rice False Smut (RFS), caused by *Ustilaginoidea virens* (Cooke) Takahashi (also known as *Villosiclava virens* Tanaka in its teleomorph form). RFS was initially identified by Cooke in 1878 in Tirunelveli, Tamil Nadu, India, and was considered a minor and sporadic disease limited to a few areas [2]. Interestingly, RFS was commonly referred to as the "Laxmi-buti" disease, symbolizing a bountiful harvest and prosperity associated with the goddess of wealth and prosperity [3].

However, over the past two decades, RFS has gained significant attention due to its outbreaks in various countries, including major rice-growing regions in India [2,4]. This disease exhibits a unique interaction mechanism between the host and the pathogen. The pathogen interferes with pollination by preventing the formation of mature pollen and invades the stigma, styles, and young ovaries of individual spikelets. This invasion leads to the formation of yellow, olive green, or blackish spore balls, resulting in the characteristic false smut symptoms [5]. Moreover, RFS can cause grain discoloration in rice, affecting grain size, type, and chemical properties such as amylose, total protein, and phenol content [6]. These factors contribute to the increasing significance of RFS as a threat to global rice production.

The use of fungicides to control RFS has drawbacks, including potential harm to grain quality, environmental pollution, and risks to human health. In light of these concerns, there has been growing interest in utilizing bioagents as a sustainable alternative. Potential bioagents belonging to the genera *Trichoderma*, *Bacillus*, and *Pseudomonas* have garnered attention for their biological control properties [7]. These bioagents have various modes of action to suppress pathogens, either directly through contact or indirectly by releasing antimicrobial compounds, metabolites, and defense enzymes. Some bioagents also act as competitors for nutrients and habitat [8]. These beneficial biocontrol agents can effectively suppress *U. virens* invasion, enhance nutrient availability to plants, trigger defense mechanisms, and regulate hormonal balance. Collectively, this integrated approach contributes to significant improvements in rice grain quality. By harnessing the beneficial activities of *Trichoderma*, *Pseudomonas*, and *Bacillus* to enhance grain quality, this sustainable disease management strategy can promote overall rice crop productivity.

2. MATERIALS AND METHODS

2.1 Fungal Pathogen and Bio-Control Agents

Pure cultures of false smut pathogen caused by *U. virens* and native bioagents (BCAs)- TAIK 1, BIK3, PIK 1 effective against major pathogens of rice were collected from ICAR- IIRR culture collections, Hyderabad, Telangana, India (Kannan et al*.,* 2021) in 2022. *Pseudomonas otitidis*-strain POPS1 was isolated as part of Ph.D. work characterized and submitted (NCBI Accession No: ON782043). Pathogenicity of the pathogen was proved according to Koch's postulates in rice TN1 cultivar.

2.2 Antagonism Assay

Bio-efficiency of the BCAs against *U. virens* was analysed using dual culture assay on PDA plates [9]. In the case of bacterial isolates, their efficacy was checked by streaking a loop full of bacteria on either side of the pathogen disc placed in the middle of the plate and incubated in BOD at 25 ± 2℃ till the pathogen grow maximum to 9 cm in the control plate upto 15 days, radial growth of pathogens was measured and recorded. Percent inhibition was calculated by the formula [10] as given below,

$$\text{Per cent inhibition (\%)} = \frac{\text{C}-\text{T}}{\text{C}} X\ 100$$

where C = colony growth in control plate (cm), T = colony growth in treated plate (cm)

2.3 *In vivo* Application of False Smut Pathogen and its Severity in Plants

Sterile TN1 seeds, susceptible to false smut, were treated with a suspension of bioagents at a rate of 10 ml/kg of seeds. The treated seeds were then incubated for 6 hours. As per the guidelines provided by the International Seed Testing Association, 2002 approximately 25 seeds were placed on each petri plate and incubated at a temperature of 28 ± 2°C for a duration of 10 days [11]. Distilled water-treated seeds were used as a negative control in this experiment. After 25 days, the seedlings were transplanted into pots measuring 30 × 25 cm. Each pot contained 5-7 kg of autoclaved soil. Following transplantation, 30 days later, the bioagents were applied to the soil at a rate of 10 ml/kg of soil, with a concentration of 2.5×10^8 colony-forming units (CFU). At 65 days after transplantation (DAT), during the booting stage of the plants, the pathogen *U. virens* was introduced by injecting a 2 ml spore suspension into the boot of each plant [12]. This experimental setup was repeated for two seasons, with each treatment being replicated three times under controlled glasshouse conditions. The environmental conditions were maintained at a relative humidity of 85% and a temperature of 30°C. To evaluate the severity of the false smut disease in rice, the Percent Disease Index was calculated using a scale ranging from 0 to 9, which was developed by the International Rice Research Institute [13].

$$\text{PDI} = \left[\frac{\text{Sum of individual disease incidence scores}}{\text{No.of panicles examined x Maximum disease incidence score}}\right] \text{x } 100$$

2.4 Analysis of Physico-chemical Properties

The harvested grains were dried and stored in a desiccator to avoid contact with air at a temperature of 20°C for a period of three (3) months before conducting the analysis of their physico-chemical properties. Throughout the entire duration of the experiment, the moisture content of the grains was carefully maintained at a level of 12-14% using grain moisture tester. In addition to the grains stored in the desiccator, another set of grain sample weighing 100 grams was stored at a temperature of 4°C. These samples were specifically used for analysing the total phenol and total protein activity present in the grains.

2.5 Physical Properties

Rice grains obtained after the hulling process, was further subjected to polishing for a duration of 60 seconds to obtain milled rice. The hulling percentage was determined by calculating the difference in weight between the grains before and after hulling. The milled rice out-turn was expressed as a percentage of the total milled rice, including both broken and unbroken grains. The broken rice was manually separated from the sample using a separator, and the head rice recovery (HRR) was calculated as the percentage of whole, unbroken polished rice obtained from the initial rough rice sample. The polished rice was then ground into rice flour using a mini grinder (Glen, India). The kernel length (KL), kernel breadth (KB), and L/B ratio of the grains were measured using a micrometer. The volumes of cooked and milled rice were determined using the water displacement method. For measuring the volume of cooked rice, 5 grams of milled rice were cooked and recorded the change in volume. Several other parameters *viz*., including gel consistency (GC), and amylose content (AC) were also measured.

2.6 Analysis of Amylose, Protein and Total Phenol Content

The amylose content (AC) in the grain samples was measured following the protocol outlined by Juliano [14]. The crude protein content was determined by analysing the total nitrogen (N) content using the Kjeldahl method and calculating the protein content using the formula N-content × 5.95. Both the amylose content and protein content were expressed as percentages. To analyze the total phenol content, 1 gram of fine defatted brown rice flour was mixed with 10 mL of ethyl alcohol and shaken for 24 hours at 50 rpm in a shaker. After centrifugation at 10,000 rpm for 20 minutes, the supernatant was collected, and the volume was adjusted to 10 mL with ethanol. The resulting extract was used to determine the total phenol content. The phenol content was measured using Folin-Ciocalteu (Merck, India) reagent, following the method described by Zilic et al. [15] with minor modifications. The total phenol content of each sample was expressed as milligrams of catechol equivalents (CE) per gram of brown rice flour.

2.7 Statistical Analysis

The experiments were conducted in a completely randomized design (CRD) and data were subjected to a one-way analysis of variance (ANOVA), using a post hoc test with Duncan's multiple range test (DMRT) at 5% ($P \leq 0.05$) significance level in OPSTAT. Three replications were maintained during each experiment.

3. RESULTS

3.1 Antagonism Assay

Native BCAs under *in vitro* conditions were evaluated for their antagonistic nature against *U. virens*. TAIK 1 showed the maximum inhibition percentage of 64.34% over the control followed by PIK 1 (59.32%), POPS 1 (42.43%), BIK 3 (50.01%) respectively (Fig. 1).

3.2 *In vivo* Application of False Smut Pathogen and its Severity in Plants

In vivo experiments conducted under glasshouse conditions indicated a significantly more decrease in PDI of false smut disease in the plants treated with consortia of bioagents (67.54 %), followed by plants treated with TAIK 1 (50.95%), over the control (Fig. 1).

3.3 Physical Properties

Physical properties of grains such as length (L), breadth (B), and L/B (Table 1) showed significant differences among the treatments between healthy and diseased plants. The length of the grain in the 'diseased' panicle was significantly ($p < 0.05$) lesser than their corresponding healthy bioagents-treated plants panicles. Whereas, the breadth reduction in 'diseased' plants over the treated plants was insignificant. The L/B ratio was increased significantly in consortia-treated plants followed by TAIK 1 treated plants over control untreated plants. Hulling, milling, and HRR% are the three major grain quality indices governing the market price of rice were presented in (Table 1). Though each attribute differed significantly within the treatments, the percent hulling in grains from consortia-treated plants showed a significantly higher value than the grains from diseased plants. The least increase in hulling percent was most pronounced in treatment RFS+ post-application of POPS 1 treated plants (0.24%) and BIK 3 (0.90%). The highest hulling percent was observed in consortia-treated plants (6.14%), followed by TAIK 1 (4.62%) and PIK 1 (4.32%) over the control plants. In case of milling diseased plants had a higher milling percentage i.e., broken grain was observed in control than BCAs treated plants. Diseased plants (72.14%) and healthy control (70.92%) followed by RFS+ post application of consortia (67.23%) displayed a dramatic increase in milling percent in grains over consortia treated (66.33%), TAIK 1 (64.12%) followed by PIK 1 (64.09%). HRR was significantly recorded highest in consortia-treated plants (15.17%) over control plants, followed by TAIK 1 treated plants (11.61%). Least changes could be observed in plants treated with BCAs followed by RFS symptom development, results indicated on par with healthy control plants, indicating that pre-treatment of BCAs has an effect on hulling, milling and HRR%. But post application of BCAs after RFS symptom development has no significant change in indicating post application of BCAs has no effect in the increase in any of these parameters.

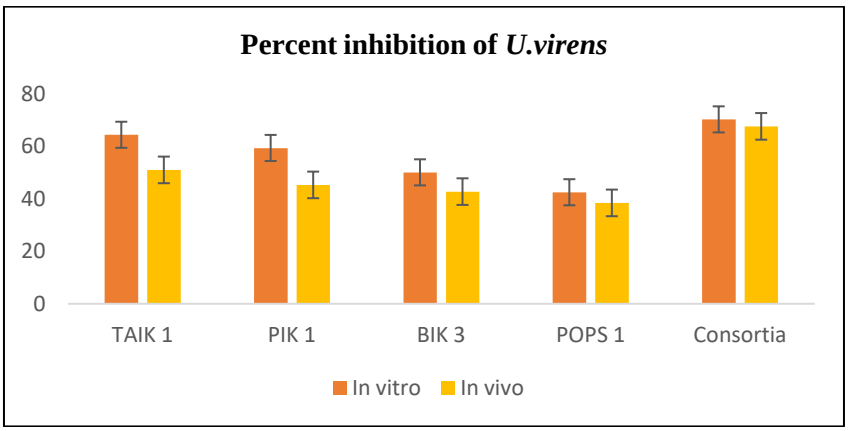

Fig. 1. Antagonism assay and *In vivo* inhibition of *U.virens* disease severity upon bioagents treatment

Table 1. Physico-chemical properties of rice grains upon bioagents treatment and false smut infection

Treatments	KL	KB	L/B	AC	GC	Hulling	Milling	HRR %	Total protein	Total phenol
T1	5.61^{b} ± 0.004	2.47^{fg} ±0.004	2.271^{b} ±0.002	23.34^{h} ± 0.004	22^{a}	67.367^{ef} ± 0.481	70.92^{b} ± 0.004	63.02^{k} ±0.004	6.24^{b} ±0.004	58.24^{q} ±0.004
T2	5.75^{a} ±0.004	2.603^{abc} ±0.002	2.209^{cd} ± 0.001	27.443^{a} ± 0.010	22^{a}	71.927^{a} ±0.274	66.33^{d} ± 0.004	69.33^{a} ±0.004	6.34^{a} ±0.004	68.28^{c} ±0.004
T3	5.657^{b} ±0.002	2.583^{abcd} ±0.002	2.187^{cde} ± 0.002	26.957^{b} ± 0.002	22^{a}	68.5^{c} ± 0.341	64.12^{h} ± 0.004	67.23^{c} ±0.004	6.283^{b} ±0.002	64.34^{i} ±0.004
T4	5.65^{b} ±0.004	2.523^{ef} ± 0.002	2.239^{bc} ± 0.002	25.667^{d} ± 0.002	22^{a}	67.047^{fg} ± 0.024	64.02^{ij} ± 0.004	65.33^{f} ±0.004	6.263^{b} ±0.002	62.12^{o} ±0.004
T5	5.64^{b} ±0.001	2.527^{def} ± 0.002	2.232^{bc} ± 0.002	26.567^{c} ± 0.002	22^{a}	67.62^{def} ± 0.004	64.09^{hi} ± 0.004	66.13^{e} ±0.004	6.27^{b} ±0.002	63.45^{k} ±0.004
T6	5.627^{b} ±0.002	2.553^{cde} ±0.002	2.204^{cd} ± 0.001	24.843^{f} ± 0.002	22^{a}	67.68^{def} ± 0.004	64.05^{ij} ± 0.004	64.46^{h} ±0.004	6.243^{b} ±0.002	61.23^{p} ±0.004
T7	5.43^{e} ± 0.002	2.63^{a} ±0.004	2.065^{f} ±0.003	23.197^{h} ±0.002	22^{a}	65.12^{j} ±0.004	72.14^{a} ±0.004	59.33^{op} ±0.004	5.82^{e} ±0.004	64.56^{g} ±0.004
T8	5.543^{c} ±0.002	2.483^{f} ±0.002	2.232^{bc} ±0.002	25.513^{d} ±0.002	22^{a}	68.13^{cd} ±0.004	65.67^{e} ±0.004	66.22^{d} ±0.004	5.94^{d} ±0.004	66.34^{d} ±0.004
T9	5.52^{cd} ±0.002	2.48^{fg} ±0.004	2.226^{bcd} ±0.005	25.033^{ef} ±0.002	22^{a}	67.24^{f} ±0.004	65.02^{g} ±0.004	64.32^{i} ±0.004	5.863^{e} ±0.002	64.12^{j} ±0.004
T10	5.48^{de} ±0.002	2.563^{bcde} ±0.002	2.138^{e} ±0.001	25.19^{e} ±0.001	22^{a}	67.93^{cde} ±0.004	65.09^{f} ±0.004	65.14^{g} ±0.004	5.92^{d} ±0.004	65.45^{e} ±0.004
T11	5.783^{a} ±0.002	2.42^{g} ±0.004	2.39^{a} ±0.005	23.67^{g} ±0.002	22^{a}	67.02^{fg} ±0.004	65.05^{fg} ±0.004	63.46^{j} ±0.004	5.85^{e} ±0.004	63.23^{l} ± 0.004
T12	5.523^{cd} ±0.002	2.547^{cde} ±0.002	2.169^{de} ±0.003	25.137^{e} ±0.002	22^{a}	69.12^{b} ±0.004	67.23^{c} ±0.004	68.33^{b} ±0.004	6.02^{c} ±0.004	70.28^{a} ±0.004
T13	5.45^{e} ±0.002	2.633^{a} ±0.002	2.07^{f} ±0.002	23.37^{h} ±0.004	22^{a}	66.13^{hi} ±0.004	64.12^{h} ±0.004	59.37^{o} ±0.004	5.82^{e}±0.004	65.34^{f} ±0.004
T14	5.44^{e} ±0.002	2.63^{a} ±0.004	2.068^{f} ±0.001	23.31^{h} ±0.004	22^{a}	65.707^{ij} ±0.102	64.02^{j} ±0.004	59.47^{n} ±0.004	5.52^{f}±0.004	63.12^{m} ±0.004
T15	5.46^{e} ±0.002	2.62^{ab} ±0.004	2.084^{f} ±0.003	23.33^{h} ±0.004	22^{a}	66.02^{hi} ±0.004	64.09^{hi} ±0.004	59.57^{m} ±0.004	5.62^{g}±0.004	64.45^{h} ±0.004
T16	5.46^{e} ±0.002	2.64^{a} ±0.004	2.068^{f} ±0.005	23.297^{h} ±0.002	22^{a}	65.273^{j} ±0.107	64.05^{ij} ±0.004	59.27^{p} ±0.004	5.42^{h}±0.004	62.23^{n} ±0.004
T17	5.477^{de} ±0.002	2.633^{a} ±0.002	2.08^{f} ±0.001	23.39^{h} ±0.004	22^{a}	66.56^{gh} ±0.004	66.33^{d} ±0.004	59.67^{l} ±0.004	5.99^{i}±0.004	69.28^{b} ±0.004

Treatment details: T1= Control- only rice, T2= Consortia, T3= TAIK1, T4= BIK3, T5=PIK1, T6=POPS1, T7=pathogen control, T8= TAIK1 Seed treatment + soil application + foliar spray + RFS, T9= BIK3 Seed treatment + soil application + foliar spray + RFS, T10= PIK1 Seed treatment + soil application + foliar spray + RFS, T11= POPS1 Seed treatment + soil application + foliar spray + RFS, T12= Consortia Seed treatment + soil application + foliar spray + RFS, T13= RFS + TAIK1, T14= RFS + BIK3, T15= RFS + PIK1, T16= RFS + POPS1, T17= RFS + Consortia

Among the treatments upon application of bioagents or pathogen-infected panicles doesn't show any significant change in gel consistency (GC), which indicates cooking quality and stickiness of grain was also not changed upon RFS infection to plants (Table 1).

3.4 Chemical Properties

There were significant differences in AC, total protein% and total phenol of grains among the treatments within bioagents treated and untreated plants. AC content increased in BCAs treated plants followed by healthy control plants, diseased panicles showed reduction in amylose content, but in case of pre-treatment of bioagents followed by RFS infection there was a decrease in AC content but it was coinciding with healthy untreated plants, but less than consortia treated plants. In bioagents treated plants there was an increase in protein content, in comparison with diseased plants (Table 1). In contrast total phenol content was recorded to be highest in the case of disease plants followed by bioagents +RFS treatment.

4. DISCUSSION

The host-pathogen interaction in false smut disease of rice is a dynamic and constantly changing process, making it challenging to accurately estimate yield loss. However, by examining the parameters affected by the disease, the extent of the loss can be estimated. Previous studies on grain quality parameters affected by false smut disease are limited [16,17]. Therefore, in this study, we aimed to evaluate the changes in both the physico-chemical properties of the grain upon biocontrol agent (BCA)-treated plants affected by *U. virens* infection.

The selected BCAs, including POPS1, PIK1, BIK3, and TAIK1, showed promising results in suppressing *U. virens* both *in vitro* and *in vivo*. The suppression of the pathogen by these bacterial bioagents, such as POPS1, PIK1, and BIK3, was attributed to the secretion of antimicrobial compounds like bacteriocins, phenazines, and hydrocynaides into the growth media, inhibiting the growth of the pathogen [18,19]. *Trichoderma* spp. were found to act through competition for nutrients, production of enzymes such as chitinases, proteases, pectinases, and glucanases that degrade pathogen cell walls, and the production of antimicrobial metabolites such as Trichoviridin and Trichodermin [20].

In vivo studies demonstrated the effective suppression of *U. virens*, resulting in a significant reduction in the PDI compared to the control. When BCAs were applied before false smut inoculation, the severity of the disease decreased, and the number of smut balls was reduced. This reduction in disease severity could be attributed to the induction of systemic resistance (ISR) by these BCAs, which reside inside plant tissues as endophytes and stimulate the plant's defense mechanisms. This regulation of defense enzyme production enables the plant to effectively combat the invading pathogen [21]. Application of TAIK1 post-symptom development of false smut showed complete death of mycelia and chlamydospores within 48 hours, preventing disease spread [22].

The effect of BCAs on the quality parameters of rice grains was investigated in this study. These quality parameters include grain size, grain type, grain chalk, cooking quality, Hulling, milling, HRR and chemical properties like amylose, total protein and phenol content. Among the treatments consortia treated plants showed overall increase in the quality of grains over the negative control by 5-10 % based on the parameter considered. And also application of BCAs prior to symptom development of false smut disease would result in significant increase in quality on par with positive control. Post symptom application of BCAs also resulted in slight increase but not in a significant change. This increase in quality parameters upon BCAs could be reduction of invading pathogens development and multiplication further disease spread is overall stopped. BCAs enhance nutrient availability for the plants, which positively affects grain size, type, and nutritional composition [16]. Overall, the findings suggest that the application of BCAs can have a positive impact on the quality parameters of rice grains. It not only improves grain quality but also reduces the reliance on chemical inputs, leading to safer and more sustainable grain production.

5. CONCLUSION

In conclusion, the application of biocontrol agents (BCAs) in managing false smut disease of rice has shown promising results. BCAs such as TAIK1, PIK1, BIK3, and POPS1 have demonstrated their ability to suppress the pathogen *U. virens* through the secretion of antimicrobial compounds and competition for nutrients. *In vivo* studies revealed that BCAs effectively reduced disease severity and smut ball formation. Furthermore, the use of BCAs

resulted in improved grain quality parameters, including size, type, and nutritional composition, compared to the negative control. Application of BCAs before symptom development showed the greatest impact on grain quality, while the post-symptom application had a slight effect. Overall, the findings support the potential of BCAs in enhancing grain quality and reducing reliance on chemical inputs for safer and more sustainable rice production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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