Grain Discoloration Disease of Rice: An Emerging Threat to The Quality Food Production

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Rice is an important food crop in the world, including in India. Regardless of holding the largest area under rice, in India, the productivity is very low compared to the other rice-growing nations of the world. Among the significant reasons for the lower productivity, biotic stresses due to insect pests and diseases cause substantial yield losses every year. In recent times, rice cultivation is facing an outbreak of several emerging diseases, such as false smut and grain discoloration. Grain discoloration (GD) of paddy, popularly known as glume discoloration/dirty green complex, is an emerging biotic stress of rice in all rice-growing regions of India, threatening rice productivity and its quality. The GD has highly complex etiology in tropical and subtropical countries; it is associated with several fungi and bacterial diseases of rice such as sheath rot (*Sarocladium oryzae*), panicle blast (*Magnaporthe grisea*), brown spot (*Helminthosporium oryzae*), sheath brown spot (*Pseudomonas fuscovaginae*), grain rot (*P. glumae*), bacterial sheath rot (*P. fuscoaginae*, *P. glumae* & *P. syringae* pv. *syringae*), and bacterial brown stripe (*Acidovorax avenae* subsp. *avenae*). However, the complete pathogen profile and their dynamics at different stages of disease development in different paddy-growing ecosystems of India are not understood properly. Therefore, it is very much necessary to characterize the different pathogens and their dynamics during the disease development in selected paddy ecosystems.

This disease is widespread in temperate and tropical areas and more prevalent in areas with low temperature (and humidity) during rice booting and heading stages. The disease is especially apparent during the rainy season, and the intensity of infection varies from mild to severe. Several pathogenic bacteria have been associated with the cause of this disease in various parts of the world, such as Asia, Africa, Latin America, Europe, and Australia. The bacterial sheath rot of rice caused by *Pseudomonas oryzicola* was first described in 1955; later, the name changed to *P. syringae* pv. *syringae*. *Pseudomonas fuscovaginae*, which causes sheath brown rot, was first reported. *Burkholderia glumae* (the causal agent of both seedling and grain rot) and *Acidovorax avenae* subsp. *avenae* (A causal agent of the bacterial stripe) have been reported to be associated with this disease. In addition to these bacterial pathogens commonly reported to be involved in sheath rot complex and grain discoloration, bacteria with similar characteristics to *Pseudomonas marginalis*, *Pseudomonas fluorescens*, *Pseudomonas corrugata*, *Pseudomonas aurofacience*, *P. fluorescens* and *P. fluorescens* has been reported pathogenic and was able to induce sheath rot of rice.

In Iran, a few surveys were made in paddy fields of Mazandaran province (the major rice-growing region) to determine the causal agents of this complex disease. In one study, some fungi, including *S. oryzae*, *Cochiobolus miyabeanus*, *A. padwichii*, and Fusarium spp., were confirmed to be associated with this disease. The fungi that are reported to be associated with discoloration of grains are *Bipolaris oryzae*, *A. padwickii*, *Pyricularia oryzae*, *F. moniliforme*, *F. graminearum*, *Nigrospora oryzae*, *Epicoccum nigrum*, *Curvularia* spp., *Phoma sorghina*,

Dichotomophthoropsis nymphacearum and *Heterosporium echinunulatum* etc. The diversity and population size of phyllosphere prokaryotic will play a significant role in plant defense against pathogens. Although it has been reported in many publications, the key drivers of phyllomicrobiome composition and their functions are not completely understood. As per the previous reports, Proteobacteria, Firmicutes, Bacteroides, and Actinobacteria are the predominant phyllomicrobiome, which includes the bacterial genera such as *Kineococcus*, *Hymenobacter, Acinetobacter, Bacillus, Citrobacter, Curtobacterium, Enterobacter, Erwinia, Frigoribacterium, Methylobacterium, Pantoea, Pseudomonas*, and *Sphingomonas*.

The grain discoloration disease was previously reported in Himachal Pradesh by Sharma and Vaid in 1985. The association of pathogenic fungal microflora causing grain discoloration was reported in India. Ash-gray discoloration (*Alternaria alternata*), light brown discoloration (*S. oryzae*), black, dark purple, dark brown discoloration (*Bipolaris oryzae*), and light pink discoloration (*Fusarium* spp.) have been reported on the stored paddy seeds leading to the abnormal seedling growth and systemic infection leading to the diseases at the later stage of the crop. The association of eight fungal genera (*Curuvlaria lunata*, Alternaria, *Helminthosporium oryzae*, *Drechslera oryzae*, *C. affinis*, *S. oryzae*, *Aspergillus niger*, Fusarium, *M. salvini*) was reported in the discolored grains of paddy collected from rice field during harvesting. The incidence of grain discoloration in all the rice-growing districts of Northeastern Karnataka ranged from 3.17-78.36%. *Pantoea ananatis* was reported to be causing new blight disease of paddy in India. This pathogen has been reported to be associated with grain discoloration of rice in China, leaf blight and bulb decay of onion in the United States, and leaf blight of rice in Korea. As the pathogen has already been included as an agent causing the grain discoloration disease in China, its association with the Indian condition needs to be ascertained. Recently, many fungal genera, including both pathogenic and saprophytic species such as *A. flavus*, *A. niger*, *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., *Curuvlaria* spp., *Rhizopus* spp., *Cercospora* spp., *Trichoderma* spp., *Chaetomium* spp., *P. oryzae*, *H. oryzae*, *S. oryzae* and other unidentified fungal genera were reported.

Although many fungi (Pathogenic & Non-pathogenic) and bacteria have been reported from the diseased sample in India, the etiology remains unclear. The dynamics of pathogens during the different stages of disease development/panicle development are not studied in any part of the world. This information is crucial for designing effective management strategies. The management of this disease is trickier as minimum information is available on disease etiology. It is heavily dependent on fungicides/bactericides but with limited success due to a lack of information on the pathogen's profile and its dynamics. Many reports where fungicides have been recommended for controlling this disease. However, fungicides are not compatible with the environment and trade due to their long persistence in the environment and grain. Many of the useful fungicides, such as Carbendazim, have been banned or recommended for the ban shortly. Therefore, the search for effective and eco-friendly strategies will assume significance. Also, the time of appearance of the disease (appears after the grain filling stage) provides relatively no opportunity period for the farmers to take up the control measures. Moreover, many of the reported pathogens are toxigenic

(*Aspergillus*, *Curvularia*, *Alternaria*, etc.,) and the pathogen-specific toxin produced on grain and their impact on rice grain quality is poorly understood. The difficulty in ascertaining the pathogen profile in the past was mainly due to the non-availability of robust and accurate techniques such as advanced genomic tools. Presently, metagenomic-based NGS (mNGS) tools are available. It can be employed to characterize all the associated pathogens and core-phyllo-microbiome in the diseased samples in the shortest possible time. Combining this mNGS with conventional microbiological tools can give an accurate count and diversity of the microbiome.

The disease affects all the cultivated rice varieties in all rice-growing regions in both Kharif and summer seasons. Presently, the minimum threshold for the procurement of discolored grain is kept at 3%, and all the samples above that are either rejected or procured at a lower price. Moreover, reports on the association of toxicogenic fungi (*Aspergillus* spp. and *Fusarium* spp.) in the discolored grains pose more concern for human and animal health. At present, only fungicides/bactericides have been recommended for controlling the diseases. However, spraying during grain maturity to the harvesting stage leads to more accumulation of fungicide residue in the grain, affecting international trade and the health of domestic consumers. It has also been reported that the GD-affected seeds show poor germination and seedling vigor, leading to crop loss at the early stage of the crop when such seeds are used for sowing in the subsequent year. Therefore, it is necessary to look through all these lacunas to research the present status of grain discoloration diseases of paddy in India.

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