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RESEARCH ARTICLE

Screening of rice (*Oryza sativa* L.) cultivars for resistance against the grain discolouration disease complex

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Abstract

Grain discolouration is an emerging complex fungal disease of rice affecting the rice glume and kernel. Recently, it has become a severe menace throughout India, affecting the grain quality and yield. Hence, there is an urgent need to identify the resistance source and devise standard integrated management practices. Given this scenario, a pot culture experiment was conducted in a glasshouse to screen Kerala Agricultural University (KAU) released seventeen rice varieties, namely, Bhadra, Asha, Karthika, Aruna, Makom, Pavithra, Panchami, Uma, Karishma, Krishnanjana, Gouri, Prathyasa, Shreyas, Pournami, Athira, Matta Triveni and Annapoorna for resistance against grain discolouration disease of rice. The statistical design followed was a completely randomised design (CRD) with seventeen treatments (varieties) and three replications. Artificial inoculation was done at the booting stage of the crop using the spore suspension injection method. Resistance levels among the varieties were evaluated based on the estimated disease parameters such as percent panicle infection (disease incidence), spikelet infection, grain discolouration and percent disease index (disease severity). Among the assessed disease parameters, the highest grain discolouration (59.39 %) and disease severity (56.39 %) were observed in the case of the susceptible check, Uma. The lowest grain discolouration (17.18 %) and disease severity (25.69 %) were observed in the case of the moderately susceptible variety, Shreyas (MO 22). None of the varieties came out to be immune or resistant. The entire study revealed that varieties, MO 22 (Shreyas) and MO 21 (Prathyasa), were moderately susceptible and the rest were susceptible or highly susceptible.

Keywords: disease complex; glume discolouration; grain discolouration; host plant resistance; resistance; rice disease

Introduction

Rice (*Oryza sativa* L.) serves as a major staple food source for more than half of the world's population and is cultivated worldwide (1). Rice crop is affected by various fungal, bacterial and viral diseases. Among the fungal diseases, blast, sheath blight, sheath rot, brown spot, grain discolouration and false smut are the major ones which lead to yield loss. Even though efforts have been made to intensify rice production by introducing improved varieties and production technologies, grain yield has sharply declined in recent years (2). The increasing demand for rice due to population rise and urbanisation highlights the need to enhance rice production in the future.

Grain discolouration is an emerging yield-reducing disease that threatens rice crops (3). It is also known as "glume discolouration" or "dirty panicle". Over 59 fungal genera and 99 species are reported to be associated with the rice seeds. The most important ones are *Bipolaris oryzae*, *Alternaria padwickii*, *Fusarium moniliforme*, *F. oxysporum*, *Curvularia lunata* and *Aspergillus* spp. (4). Symptoms of rice grain discolouration include distinct spots on grains ranging from brown to black. The direct effects of grain discolouration can be seen on the morphology of grains in terms of size, colour and shape. In other cases, there will be black blotches, which may be flecks which are large enough to cover the inter-glume space. Formation of brown immature lighter grains, kernel discolouration, glume discolouration and grain rot are also the symptoms of this disease complex (5). Various methods, reported by earlier workers, can be used to manage this disease. Growing resistant varieties, early detection and management of the disease can overcome the heavy yield loss (6).

Grain discolouration disease is widespread in major rice -growing districts of Kerala, affecting grain yield and quality. Even though a few studies on managing grain discolouration disease have been conducted, no systematic studies have been conducted to precisely identify the associated pathogens and resistant sources against the disease (7). Given this scenario, the present study was proposed to screen the rice cultivars released by KAU for resistance against the grain discolouration disease.

Materials and Methods

A pot culture experiment was conducted in the glass house of the Department of Plant Pathology, College of Agriculture, Vellayani during September 2023 to screen seventeen KAU rice

varieties namely, Krishnanjana (MO 19) (V1), Pournami (MO 23) (V2), Asha (MO 5) (V3), Karishma (MO 18) (V4), Uma (MO 16) (V5), Karthika (MO 7) (V6), Sreyas (MO 22) (V7), Prathyasa (MO 21) (V8), Bhadra (MO 4) (V9), Athira (PTB 21) (V10), Aruna (MO 8) (V11), Pavithra (MO 13) (V12), Matta Triveni (PTB 45) (V13), Panchami (MO 14) (V14), Gouri (MO 20) (V15), Annapoorna (PTB 35) (V16) and Makom (MO 9) (V17) for resistance against virulent genera of grain discolouration pathogens. The variety Uma (MO 16) was maintained as the susceptible check. The statistical design followed was a CRD with 17 treatments and 3 replications. Healthy rice seeds were sown in micro planter pots filled with sterile paddy field soil. Twelve-day-old seedlings were transplanted into the plastic planter pots filled with paddy field soil, sand and dry cow dung in a 2:1:1 proportion. Crop management in terms of cultural operations and fertilization was followed as per the package of practices of Kerala Agricultural University (8). Inoculation was done at the booting stage of the crop using the spore suspension injection method.

Spore suspensions of *Microdochium fisheri*, *F. moniliforme* and representative virulent isolates from predominant genera of the pathogens, including *Curvularia* spp., *Bipolaris* spp. and *Fusarium* spp., were prepared using seven-day-old cultures. Ten mL of sterile water was poured on the culture plate and was gently smeared with a glass rod without disturbing the agar. The suspension was filtered through sterile cheesecloth and the filtrate was measured for conidial concentration using the haemocytometer and adjusted to 10^5 conidia mL^{-1}. Further, the individual spore suspensions were mixed and injected into the rice boots and the plants were covered with a moistened polythene cover to maintain humidity and kept for symptom development. The observations, such as nature of symptoms, number of panicles infected, number of spikelets affected and number of grains infected were recorded for each variety. Disease scoring was also done using the IRRI-SES scale based on percent grain discolouration to compute per cent disease index (9) (Table 1). Further, percent grain discolouration was used to categorise the varieties based on resistance using the IRRI-SES disease scoring scale.

Disease parameters namely, panicle infection percent, spikelet infection percent, grain discolouration percent and percent disease index were calculated using the following formulae.

Panicle infection (%) =

$$\frac{\text{Number of infected panicles}}{\text{Total number of panicles}} \times 100$$

Spikelet infection (%) =

$$\frac{\text{Number of infected spikelets}}{\text{Total number of spikelets}} \times 100$$

Grain discolouration (%) =

$$\frac{\text{Number of discoloured grains}}{\text{Total number of grains}} \times 100$$

Disease index (%) =

$$\frac{\text{Sum of individual diseased grain ratings}}{\text{Number of grains assessed} \times \text{Maximum disease score}} \times 100$$

Table 1. Disease scoring chart (IRRI-SES scale)

Disease score	Description
0	No symptom of discolouration
1	Less than 1 % discolouration
3	1-5 % discolouration
5	6-25 % discolouration
7	26-50 % discolouration
9	51-100 % discolouration

Results and Discussion

A pot culture experiment was conducted to screen 17 KAU rice varieties for resistance against grain discolouration disease of rice. Artificial inoculation was done at the booting stage by using the spore suspension injection method. A general view of the pot culture experiment and steps in screening rice varieties for resistance is shown in Fig. 1.

The nature of symptoms observed in the case of 17 different varieties is given in Table 2. The panicle infection percent, spikelet infection percent, grain discolouration percent and percent disease index are computed and presented in Fig. 2. The symptoms observed among different rice varieties are highlighted in Fig. 3.

Among the 17 varieties, Uma (MO 16) (standard susceptible check) showed the highest panicle infection (100 %), spikelet infection (84.66 %), grain discolouration (59.39 %) and disease index (56.39 %) compared to other varieties. Annapoorna variety (PTB 35) showed on-par reactions with Uma in terms of panicle infection (100 %), spikelet infection (86.85 %) and grain discolouration (58.24 %).

Shreyas (MO 22) significantly showed the least panicle infection (48.75 %), spikelet infection (47.47 %), grain discolouration (17.18 %) and disease index (25.69 %), followed by Prathyasa (MO 21), showed panicle infection of 54.87 %, spikelet infection of 53.09 %, grain discolouration of 19.84 % and disease index of 29.56%.

The screened varieties were categorised into immune, resistant and susceptible based on grain discolouration percent using the IRRI-SES disease scoring chart (Table 3). Out of the 17 varieties screened, no variety was immune, resistant or moderately resistant. The varieties MO 22 and MO 21 were moderately susceptible, with grain discolouration percent and disease scores of 6-25 % and five, respectively.

A previous study reported the variation in disease incidence (25-92 %) and severity among rice varieties due to grain discolouration. They screened 37 rice varieties for resistance against grain discolouration and reported that none were immune or resistant. Varieties, Dhala Heera, Ratna and Khitish were moderately susceptible and the rest of the varieties were susceptible or highly susceptible (10). Another study reported the variations in the grain discolouration percent and disease severity among 37 rice varieties screened against the grain discolouration disease. Among them, none were found to be immune or resistant, but four genotypes (BR-2655, Jaya, KCP-1, Rajamudi and Ratnachudi) were moderately resistant, 21 genotypes were moderately susceptible and 12 genotypes were susceptible (11). In yet

Table 2. Nature of symptoms exhibited by various rice varieties following artificial inoculation of grain discolouration pathogens

S. No.	Variety	Nature of symptoms
V1	Krishnanjana (MO 19)	Dark brown spots on glumes; dark brown and ash grey discolouration of glumes with seed deformation.
V2	Pournami (MO 23)	Dark brown irregular spots on glumes; dark brown, black, and ash grey discolouration of glumes; and grain chaffiness.
V3	Asha (MO 5)	Light brown, dark brown, and black discolouration of glumes; and grain chaffiness.
V4	Karishma (MO 18)	Dark brown, slightly elliptical, and circular spots on glumes; dark brown and ash grey discolouration of glumes.
V5	Uma (MO 16)	Rusty brown pin-prick-like and slightly elliptical spots on glumes; dark brown and black discolouration of glumes; and grain chaffiness.
V6	Karthika (MO 7)	Rusty brown irregular spots on glumes; dark brown, light brown, and black discolouration of glumes; and seed deformation.
V7	Shreyas (MO 22)	Dark brown circular and slightly elliptical spots on glumes; dark brown and black discolouration of glumes with white mycelia; and grain chaffiness.
V8	Prathyasa (MO 21)	Dark brown irregular spots on glumes; dark brown, ash grey, and black discolouration of glumes partially and completely.
V9	Bhadra (MO 4)	Dark brown, light brown circular and slightly elliptical spots on glumes; light brown, dark brown, and light pink discolouration of glumes with white mycelia.
V10	Athira (PTB 21)	Eye-shaped spots on glumes with dark brown margin and ash grey centre, circular and slightly elliptical spots on glumes, dark brown and black irregular discolouration of glumes; and grain chaffiness.
V11	Aruna (MO 8)	Dark brown pin-prick-like circular spots on glumes, dark brown, black, and ash grey discolouration of glumes and grain chaffiness.
V12	Pavithra (MO 13)	Dark brown irregular spots on glumes; dark brown, black, ash grey, and light pink discolouration of glumes; and seed deformation.
V13	Matta Triveni (PTB 45)	Dark brown irregular spots on glumes; dark brown discolouration of glumes; and seed deformation.
V14	Panchami (MO 14)	Light brown slightly elliptical and irregular spots on glumes; light brown, dark brown, and black discolouration of glumes; and grain chaffiness.
V15	Gouri (MO 20)	Dark brown circular spots on glumes, light brown, dark brown, and ash grey discolouration of glumes; and grain chaffiness.
V16	Annapoorna (PTB 35)	Dark brown irregular spots on glumes, light brown, dark brown, and ash grey discolouration of glumes; and seed deformation.
V 17	Makom (MO 19)	Dark brown and light brown circular and irregular spots on glumes, light brown, dark brown, and black discolouration of glumes; and grain chaffiness.

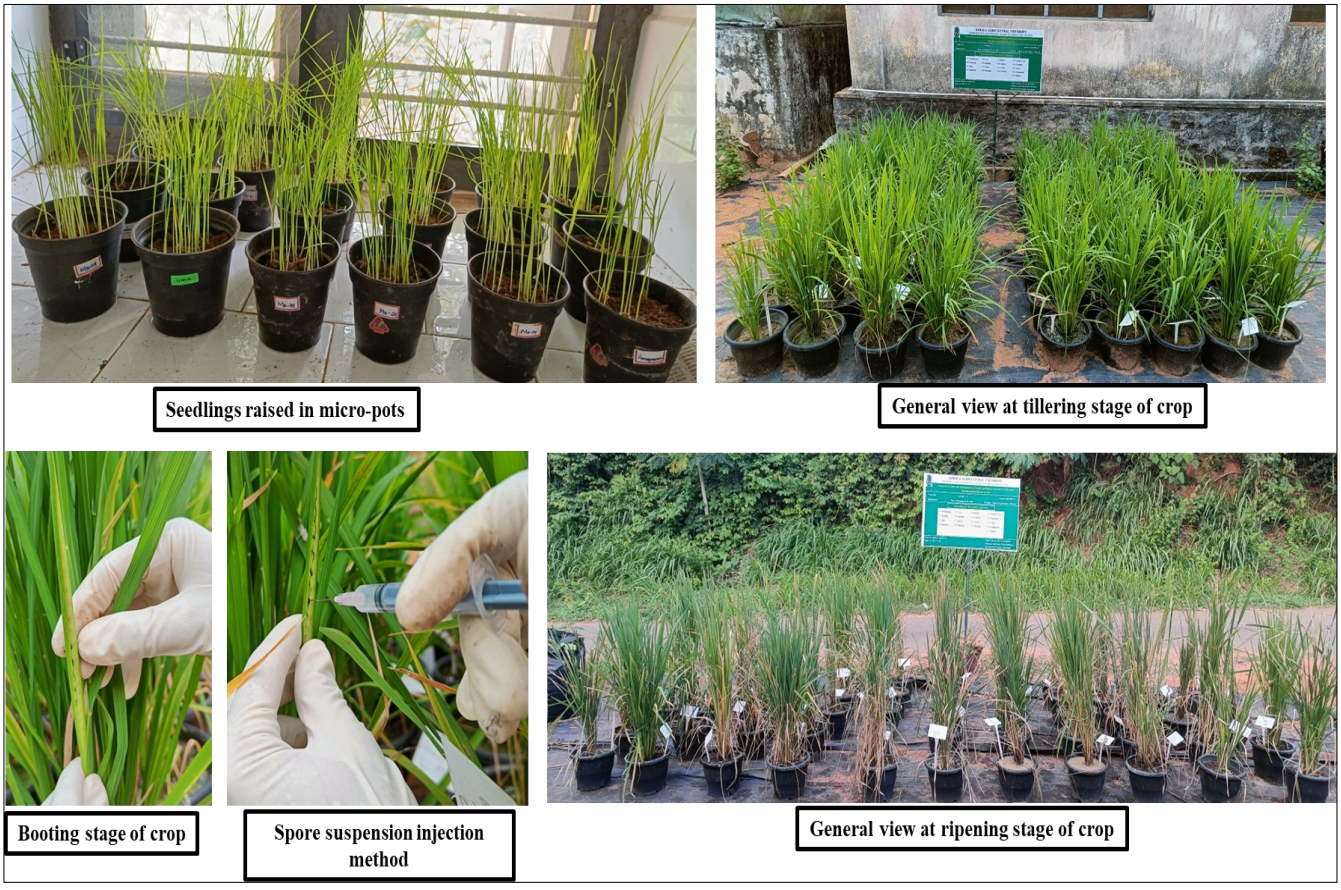

Fig. 1. General view of the pot culture experiment and steps followed in screening rice varieties for resistance.

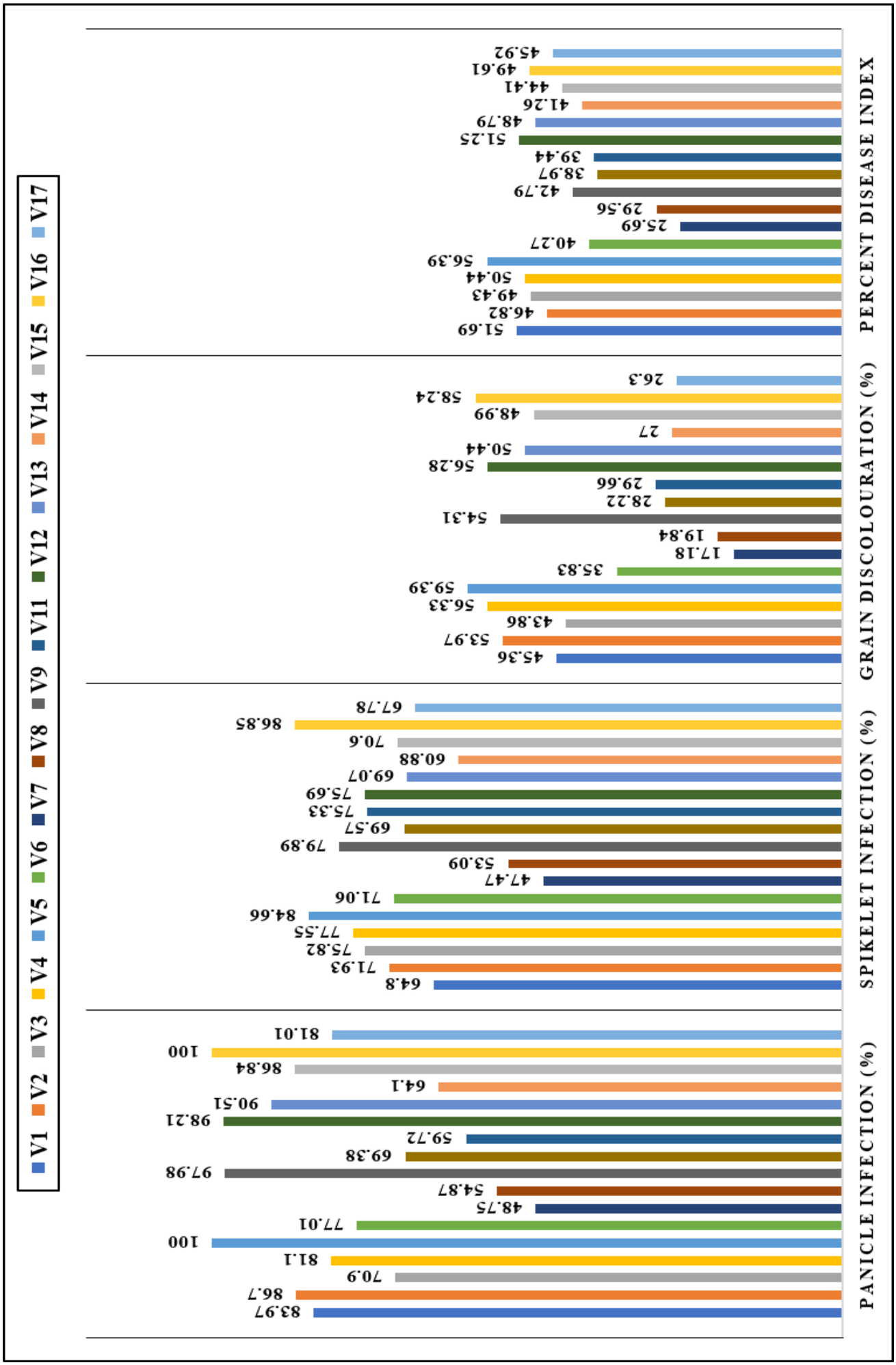

Fig. 2. Response of the rice cultivars to grain discolouration pathogens in terms of variations in the assessed disease parameters.

Fig. 3. Symptoms of grain discolouration observed in different rice cultivars following artificial inoculation.

Table 3. Categorisation of screened rice varieties based on grain discolouration percent using the IRRI-SES scale

Disease score	Description	Reaction	Varieties
0	No symptom of discolouration	Immune	NIL
1	Less than 1 % discolouration	Resistant	NIL
3	1-5 % discolouration	Moderately resistant	NIL
5	6-25 % discolouration	Moderately susceptible	MO 22, MO 21
7	26-50 % discolouration	Susceptible	MO 19, MO 5, MO 7, PTB 21, PTB 45, MO 14, MO 8, MO 20 and MO 9.
9	51-100 % discolouration	Highly susceptible	MO 23, MO 18, MO 16, MO 4, MO 13, PTB 35

another study, 60 rice genotypes were screened against the grain discolouration disease of rice. Out of these, none of the genotypes were immune; three genotypes, namely, IET- 24486, IET- 25654 and IET- 25676, showed a resistant reaction, fourteen genotypes were moderately resistant and 35 genotypes were moderately susceptible (12).

The variation of the symptoms and disease parameters among the rice varieties may be mainly attributed to the genetic composition of the host, the nature of the defense mechanism triggered, environmental factors, soil factors and nutritional status. The high incidence of disease and the unavailability of a resistance source may be attributed to the emerging and complex nature of the disease, which can confuse the host plant and delay the activation of defence responses due to the involvement of multiple virulence factors in combination by the associated pathogens. Moreover, host susceptibility in the case of co-infection is mainly attributed to the pathogen-triggered host plant susceptibility, competition among the co-infecting pathogens and cooperation among the co-infecting pathogens for invasion, establishment and nutrient acquisition (13).

Conclusion

The outcome of this study highlights the absence of immune, resistant or moderately resistant rice cultivars against the grain discolouration disease among the screened ones. Interestingly, two cultivars, MO 22 and MO 21 were moderately susceptible. As there are no detailed etiological studies, resistance sources and efficient integrated management strategies to tackle the menace, there is scope to shed light on these untouched areas in future.

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Authors' contributions

APK carried out the experiment, analysed data and wrote draft of the manuscript. SM designed the study, supervised and provided the critical comments. AB aided in data analysis and interpretation. RNV provided critical comments. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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