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Screening of rice variety for resistance against sheath blight caused by *Rhizoctonia solani*

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Abstract
An experiment was laid out during 2017-2018 at experimental field of IGKV, Raipur and detailed observation of sheath blight of rice was taken. The disease samples of rice were collected from farmer fields of different places like Raipur, Durg, Rajnandgaon and Gariyaband. The fungus *Rhizoctonia solani* were isolated and purified in the laboratory. Pathogenicity of the isolates was determined on susceptible rice cultivar Swarna and Kranti under the field condition. Host plant resistance were also studied under the field condition. Screening of rice entries of Kranti Variety against four isolates of *Rhizoctonia solani* collected from different places of Chhattisgarh i.e. Raipur, Durg, Rajnandgaon and Gariyaband. Twenty three entries of Kranti variety i.e., NSN-1-77, NSN-1-83, BRIDING-4, GIP-108, GIP-855, NSN-1-89, NSN-1-79, NSN-1-107, NSN-2-68, NSN-2-151, NSN-2-298, NSN-2-329, NSN-1-5, NSN-2-86, DSN-112, SIET-1-42, SIET-2-55, FSVT-MS-18, CANP-30, NSN-1-116, NSN-1-123, NSN-1-152, DSN-38 along with check TN1 were grown in field. Responses of plant in various entries were recorded according to Standard evaluation system (SES), IRRI (2014) and graded as per disease rating scale i.e. Immune, Highly Resistant, Resistant, Moderately Resistant, Susceptible and Highly Susceptible. In host plant resistance study under field conditions the rice variety kranti which are inoculated with *R. solani* of Gariyaband isolates i.e. NSN-1-77, NSN-1-83, BRIDING-4, GIP-108, GIP-855, NSN-1-89, NSN-1-79, NSN-1-107, NSN-2-68, NSN-2-151, NSN-2-298, NSN-2-329, NSN-1-5, NSN-2-86, DSN-112, SIET-1-42, SIET-2-55, FSVT-MS-18, CANP-30, NSN-1-116, NSN-1-152 and DSN-38 were showed resistant reaction against sheath blight disease of rice**.** Continuous evaluation of resistant plant varieties and their multiplication testing to confirm the resistance donor source for resistance to sheath blight at artificial inoculated conditions will be useful to breed a resistance variety under rice breeding programme.

Keywords: Screening, resistance against, sheath blight, *Rhizoctonia solani*

Introduction
Rice (*Oryza sativa* L.) is the second most important cereal crop and the staple food for more than half of the world's population. The production of rice to be achieved by 2020 is 128 million tonnes to feed the growing population of India. Rice provides 20% of the world's dietary energy supply followed by maize and wheat. In the world at present the area of rice is 161.10 M ha with production of 483.00 million metric tons and productivity of 2.98 Mt ha-1. In India the area of rice is 44.00 Mha with production of 103.3 million metric tons and productivity 3.59 Mt ha-1. In Chhattisgarh state rice occupies an area of 3.60 Mha-1 with the production of 6.29 Mt and productivity of 1.6 Mt ha-1. Worldwide the annual losses due to rice diseases estimated to be 10- 15%.

The Chhattisgarh state is popularly known as "rice bowl" of the country as rice is the principal crop of this state and about 70 percent of net sown area is covered under rice. Rice is attacked by number of fungal, bacterial, viral and nematode diseases. Among all pathogenic organisms, fungal pathogens are limiting the rice productivity to great extent. Serious incidences of diseases such as blast, sheath blight or bacterial blight have been reported from rice growing areas of Chhattisgarh region.

The initial symptoms usually develop as lesions on sheaths of lower leaves near the waterline when plants are in the late tillering or nearly internode elongation growth stage. These lesions usually develop just below the leaf collar as oval to elliptical, green grey, water-soaked spots about ¼ inch wide and ½ to ¼ inch long. With age, the lesions expand and the centre of the lesions may become bleached with an irregular brown border. When humidity exceeds 95% and temperature ranges from 29 to 32 ^{O}C, infection spreads rapidly by means of runner hyphae which appear on plant parts, including leaf blades, causing irregularly

shaped lesions with brown borders as bands. This symptom generally referred as "banded blight".

The fungus *Rhizoctonia solani* produced usually long cells of septate mycelium which are hyline within young, yellowish brown. It produced large number of globose sclerotia which initially turn white, late turn brown to purplish brown. Sclerotia serve as a major source of primary inoculums. Wide host range of the pathogen *Rhizoctonia solani* makes management of the disease a different test. Breeding for resistance through effective has not succeeded due to lack of suitable clones. So far complete resistance source has not been found against this fungus, mainly because resistance is governed by quantitative trait loci (QTL), i.e., controlled by polygenes. Hence, the disease is being managed by changing the cultural practices by one of chemical fungicide.

Materials and Methods

1. Collection of disease samples, isolation and purification of *Rhizoctonia solani*

1.1 Collection of disease samples

The disease samples were collected from naturally infected rice plants from farmer field of different places like Durg, Rajnandgaon, Gariyaband and under the experiment area of Plant Pathology, IGKV, Raipur during *kharif* 2017.

1.2 Isolation of pathogen

The diseased samples were washed thoroughly with tap water. Small portion of infected parts containing healthy as well as diseased tissues were cut in to 0.5 cm pieces with the help of sterilized scalpel blade. These pieces were then surface sterilized with 1 percent sodium hypochlorite solution for 1 minute with 3 subsequent changes in sterilized water to remove traces of the chemical. The pieces were then transferred aseptically to petri dishes containing sterilized Potato Dextrose Agar (PDA) and incubated at 28±2 ºC under BOD incubator. The petri dishes were examined at regular time intervals for fungal growth radiating from the infected pieces.

1.3 Purification

In each petri dish about 20 ml PDA medium was poured after supplementing with pinch of streptomycin sulphate, to avoid bacterial contamination. One 8 mm mycelial disc from a freshly isolated culture was transferred aseptically to the solidified PDA in each petri dish. The dishes were incubated at 28±2 ºC in BOD incubator. Adequate numbers of sub culture transformation were separately made for further purification.

2. Pathogenicity test

The pathogenicity of the isolates was determined by rice stem bits inoculation method on 40-day-old susceptible rice cultivar Swarna and Kranti. Four to five rice stem bits colonized with fungal mycelia (and sclerotia) are then placed in between the tillers in the central region of the hill, 5-10 cm above the water line. The inoculated plants were observed regularly for development of symptoms. For artificial inoculation, rice plants at maximum tillering stage were taken for inoculation. The inoculation was done by placing sclerotia of *Rhizoctonia solani* with the help of sterilized forceps in the centre of each hill. For each variety/entry five healthy tillers were inoculated with four isolates (Collected from Raipur, Durg, Rajnandgaon, Gariyaband) at random. After inoculation, crop was regularly watched for appearance of disease. Rice varieties/entries were screened against sheath blight severity. Each plot was observed in number of infected tiller and each tiller were observed plant height and symptoms length of sheath blight of rice. The disease development was recorded in each variety and graded as per standard evaluation system (SES), IRRI (2014) [11] presented in Table 1.

3. Mass multiplication of inoculum

Stems of 35-40 days old rice plants were cut in to pieces of about 2 cm size and filled in to 500 ml Erlenmeyer flasks upto one third. Flasks were autoclaved at 15 pound per square inch for 30 minutes. Mycelial discs of 5 mm diameter cut from the margin of 48 hrs old culture of the pathogen were inoculated into the flask and incubated at 28±2 ºC up to fifteen days for full growth of fungus and sclerotia formation. For artificial inoculation, rice plants at maximum tillering stage were taken for inoculation.

4. Screening of rice entries against four isolates of *Rhizoctonia solani* collected from Raipur, Durg, Rajnandgaon and Gariyaband.

The study was conducted in bunded rice field and under irrigated conditions during *Kharif* 2017. Twenty three rice entries of kranti variety i.e., NSN-1-77, NSN-1-83, BRIDING-4, GIP-108, GIP-855, NSN-1-89, NSN-1-79, NSN-1-107, NSN-2-68, NSN-2-151, NSN-2-298, NSN-2-329, NSN-1-5, NSN-2-86, DSN-112, SIET-1-42, SIET-2-55, FSVT-MS-18, CANP-30, NSN-1-116, NSN-1-123, NSN-1-152, DSN-38,were grown in I.G.K.V., Raipur Research field. The rice entries were shown in a nursery bed by direct sowing in simple two rows design with a spacing of 20 cm from row to row and a single row of check TN-1 was taken. Seed placement was done approximately at a distance of 2 to 3 cm. Fertilizer was applied @ N120 P50 K0 kg/ha. Fifty percent of N and total P were given as first basal dose and remaining N applied in two split doses. The environment was kept aseptic to ensure that the seedlings were disease and contaminant-free.

Table 1: Standard evaluation system (SES), IRRI (2014) [11]

Disease rating scale	Response	Description
0	Immune	No Infection
1	Highly Resistant	Vertical spread of the lesions up to 20% of plant height
3	Resistant s	Vertical spread of the lesions up to 21-30% of plant height
5	Moderately Resistant	Vertical spread of the lesions up to 31-45% of plant height
7	Susceptible	Vertical spread of the lesions up to 46-65% of plant height
9	Highly Susceptible	Vertical spread of the lesions up to 66-100% of plant height

4.1 *Rhizoctonia solani* inoculum preparation
Stems of 35-40 days old rice plants were cut in to pieces of about 2 cm size and filled in to 500 ml Erlenmeyer flasks upto one third. Flasks were autoclaved at 15 pound per square inch for 30 minutes. Mycelial discs of 5 mm diameter cut from the margin of 48 hrs old culture of the pathogen were inoculated into the flask and incubated at 27±2 ºC up to fifteen days for full growth of fungus and formation sclerotia.

4.2 Method of inoculation
For artificial inoculation, rice plants at maximum tillering stage were taken for inoculation. The inoculation was done by placing sclerotia of *Rhizoctonia solani* with the help of sterilized forceps in the center of each hill. For each variety five healthy tillers were inoculated at random. After inoculation, crop was regularly watched for appearance of disease. Rice varieties/entries were screened against sheath blight severity. The disease development was recorded in each variety and. Observations were recorded on 21 days after inoculation and graded as per 0-9 standard evaluation system (SES), IRRI (2014) [11] scale presented in Table 1.

Result and Discussion
1. Collection of disease samples, isolation, purification of *Rhizoctonia solani*
1.1 The symptoms
The sheath blight symptoms produced on leaf sheath near water level as water soaked lesions that are circular to oblong, ellipsoid, ovoid or even irregularly elongated (3x1 cm) and discoloured, that later turn into discrete lesion with pale greenish to grey to grayish white center with narrow blackish to dark brown margin. Finally, 4-5 such lesions coalesce and girdle whole leaf sheath, culm, boot and flag leaf whereby the encircled tiller dries. On leaf, sometime copper coloured transverse bands are also produced. These types of symptoms are popularly called as banded blight.

1.2 Collection of disease samples
The disease samples were collected from naturally infected rice plants from fields of farms of different places and under the experiment area of Plant Pathology, IGKV, Raipur and Durg, Gariyaband, Rajnandgaon during *Kharif* 2017.

1.3 Isolation of pathogen
The collected diseased samples were washed thoroughly with tap water. Small portion of infected parts containing healthy as well as diseased tissues were cut in to 0.5 cm pieces with the help of sterilized scalpel blade. These pieces were then surface sterilized with 1 percent sodium hypochlorite solution for 1 minute with 3 subsequent changes in sterilized water to remove traces of the chemical. The pieces were then transferred aseptically to petri dishes containing sterilized Potato Dextrose Agar (PDA) and incubated at 28±2 ºC under BOD incubator. The petri dishes were examined at regular time intervals for fungal growth radiating from the infected pieces.

1.4 Purification
In each petri dish about 20 ml PDA medium was poured after supplementing with pinch of streptomycin sulphate, to avoid bacterial contamination. One 5 mm mycelial disc from a freshly isolated culture was transferred aseptically to the solidified PDA in each petri dish. The dishes were incubated at 28±2 ºC in BOD incubator. Adequate numbers of sub culture transformation were separately made for further purification.

1.5 Identification of the test fungus
The isolated fungi were identified on the basis of following morphological characteristics. The genus *Rhizoctonia solani* belongs to Form Class Deuteromycetes that does not make vegetative spores and present as mycelium and sclerotia. It produces shade of brown hypha, constriction at the point of branching and right angle branching in matured hyphae. The isolate shared typical characteristics of *R. solani*

a) Branching at right angle near the distal septum of the cell in young vegetative hyphae.
b) Formation of a septum in the branch near the point of origin.
c) Constriction of the branch at origin, dolipore septum.
d) No clamp connection.
e) Presence of moniloid cells.
f) Undifferentiated sclerotia and
g) Absence of rhizomorphs.

Sclerotia were undifferentiated aggregations of thick-walled cells, small (1-3-mm diameter) irregular-shaped, brown to black structures (Gutierrez *et al.* 1997) [9]. Similar result on isolation, purification and identification were reported by Parmeter and Whitney (1970) [23].

2. Pathogenicity test
Under artificial inoculated conditions the sheath blight pathogen showed their pathogenic ability and produced typical sheath bight symptoms on susceptible variety swarna and Kranti after 3 days of inoculation the rice plants produced the typical symptoms such as:
Dark lesions were developed on the pseudostem (near water line). Some infected plants at the later growth stage of the plant, small dark bodies (sclerotia) developed. With the lapse of time numerous round little shining and dark brown to black color sclerotial bodies were formed on the affected sheath. Sclerotia were also noticed in the hollow internodal portion at maturity and were prominently visible when opened the infected portion.
The above symptoms were in agreement with the authentic reports made by earlier workers on sheath blight of rice (Ou, 1972) [21]. The plants inoculated at seedlings stage showed 60 percent mortality also confirms that the earlier reports made by Yaqub and Shahzad (2005).

3. Screening of rice entries against four isolates of *Rhizoctonia solani* collected from Raipur, Durg, Rajnandgaon and Gariyaband
The twenty three entries of Kranti variety were screened against sheath blight of rice by artificial inoculation with four isolates collected from Raipur, Durg, Gariyaband and Rajnandgaon districts and then the entries were recorded for highly resistance reaction. The data presented in (Table No. 2). The twenty three rice entries designated as NSN-1-779(771), NSN-1-83(772), BRIDING-4(773), GIP-108(774), GIP-855(775), NSN-1-89(776), NSN-1-79(777), NSN-1-107(778), NSN-2-68(779), NSN-2-151(780), NSN-2-298(781), NSN-2-329(782), NSN-1-5(783), NSN-2-86(784), DSN-112(785), SIET-1-42(786), SIET-2-55(787), FSVT-MS-18(788), CANP-30(789), NSN-1-116(790), NSN-1-123(791), NSN-1-152(792), DSN-38(793) and check TN 1 check were showed.

Resistant reaction (Score-3) of Raipur isolates. While the twenty one entries designated as NSN-1-779(771), NSN-1-83(772), GIP-108(774), GIP-855(775), NSN-1-89(776), NSN-1-79(777), NSN-1-107(778), NSN-2-68(779), NSN-2-151(780), NSN-2-298(781), NSN-2-329(782), NSN-1-5(783), NSN-2-86(784), DSN-112(785), SIET-1-42(786), SIET-2-55(787), CANP-30(789), NSN-1-116(790), NSN-1-123(791), NSN-1-152(792), DSN-38(793) and while the nineteen entries shown resistant reaction of Durg isolates i.e- NSN-1-77(771), NSN-1-83(772), BRIDING-4(773), GIP-108(774), GIP-855(775), NSN-1-89(776), NSN-1-79(777), NSN-1-107(778), NSN-2-68(779), NSN-2-151(780), NSN-2-298(781), NSN-1-5, NSN-2-86(783), DSN-112(784), SIET-1-42(785), FSVT-MS-18(787), CANP-30(788), NSN-1-116(789), NSN-1-152(792), DSN-38(793). The twenty two entries shown resistant reaction of Gariyaband isolates i.e. NSN-1-77(771), NSN-1-83(772), BRIDING-4(773), GIP-108(774), GIP-855(775), NSN-1-89(776), NSN-1-79(777), NSN-1-107(778), NSN-2-68(779), NSN-2-151(780), NSN-2-298(781), NSN-1-5, NSN-2-86(783), DSN-112(784), SIET-1-42(785), FSVT-MS-18(787), CANP-30(788), NSN-1-116(789), NSN-1-152(792), DSN-38(793). The fifteen entries shown resistant reaction of Rajnandgaon isolates i.e. NSN-1-779(771), NSN-1-83(772), GIP-108(774), GIP-855(775), NSN-1-89(776), NSN-1-107(778), NSN-2-68(779), NSN-2-151(780), NSN-2-298(781), NSN-1-5(783), DSN-112(785), SIET-1-42(786), FSVT-MS-18(788), CANP-30(789), NSN-1-152(792). RAIPUR isolates showed moderately resistant reaction (Score-5) in two entries i.e. Briding-4(773), FSVT-MS-18(788). Durg isolates showed moderately resistant reaction in three entries i.e. NSN-2-329(782), SIET-1-42(786), NSN-1-116(790), and Rajnandgaon isolates showed moderately resistance reaction in four entries i.e. NSN-2-86(784), SIET-2-55(787), NSN-1-116(790), DSN-1-38(793), and Gariyaband isolates showed moderately resistance reaction in one entries i.e. NSN-1-123(791). One entry was recorded as susceptible (Score-7) of Durg isolate i.e. NSN-1-123(791) and BRIDING-4 (773), NSN-1-79(777), NSN-2-329 (782), NSN-1-123 (791) were recorded as susceptible of Rajnandgaon.

The above results are accordance with the findings of Mosaddeque *et al*., (2008) [17] and screened that forty four test entries of parental lines of rice with one susceptible (BR11) and one resistance check (BRR1 dhan29) were screened against sheath blight (*Rhizoctonia solani*) of rice, The pathogenicity test were studies in the laboratory. Ten lines were resistant, 31 were moderately resistant and 3 showed susceptible reaction at maximum tillering stage.

Chandra *et al*., (2016) [6] analyzed that out of 108 germplasm, screened under natural as well as under artificial inoculated condition none of the entries were found immune or resistant. However, forty five entries under artificial inoculated condition, out of 82 entries, none of the entry was found resistant. Only two entries *viz.* Baigani black and Prasada showed moderately resistant reaction, seventeen moderately susceptible and twenty seven entries showed susceptible react.

Table 2: Screening of rice entries (Kranti Variety) against sheath blight

Entries No.	Raipur	Durg	Gariyaband	Rajnandgaon
IET NO. 771	Resistant	Resistant	Resistant	Resistant
IET NO. 773	Moderately resistant	Resistant	Resistant	Moderately resistant
IET NO. 774	Resistant	Resistant	Resistant	Resistant
IET NO. 775	Resistant	Resistant	Resistant	Resistant
IET NO. 776	Resistant	Resistant	Resistant	Resistant
IET NO. 777	Resistant	Resistant	Resistant	Susceptible
IET NO. 778	Resistant	Resistant	Resistant	Resistant
IET NO. 779	Resistant	Resistant	Resistant	Resistant
IET NO. 780	Resistant	Resistant	Resistant	Resistant
IET NO. 781	Resistant	Resistant	Resistant	Resistant
IET NO. 782	Resistant	Moderately resistant	Resistant	Susceptible
IET NO. 783	Resistant	Resistant	Resistant	Resistant
IET NO. 784	Resistant	Resistant	Resistant	Moderately resistant
IET NO. 785	Resistant	Resistant	Resistant	Resistant
IET NO. 786	Resistant	Moderately resistant	Resistant	Resistant
IET NO. 787	Resistant	Resistant	Resistant	Moderately resistant
IET NO. 788	Moderately resistant	Resistant	Resistant	Resistant
IET NO. 789	Resistant	Resistant	Resistant	Resistant
IET NO. 790	Resistant	Moderately resistant	Resistant	Susceptible
IET NO. 791	Resistant	Susceptible	Moderately resistant	Susceptible
IET NO. 792	Resistant	Resistant	Resistant	Resistant
IET NO. 793	Resistant	Resistant	Resistant	Moderately resistant
Total entries= 23/ LSI	3.17	3.34	3.08	4.04

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