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Morphological, Cultural and Sclerotial Variability among Isolates of *Rhizoctonia solani* Causal Agent of Web Blight of Soybean

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A B S T R A C T

In the present study morphological, cultural and sclerotial variability was observed among 17 isolates collected from five districts of Chhattisgarh. Lowest hyphal width was observed to be that of RS 10(2.16 µm) and highest hyphal width was observed to be that of RS 14(4.38 µm) the least angle of branching was observed to be that of RS 12(77.45˚) and the highest angle of branching was observed to be that of RS 5(95.743˚). Significant variation in distance between septa was also observed among the isolates (36.34 to 11.33 µm). Based on the observation of colony colour the different isolates were assigned into three different groups i.e. pale white, light brown and ivory. The different isolates were assigned into four different groups i.e. sparse, sparse fluffy, cottony and cottony fluffy based on texture of the colony. The number of sclerotia formed ranged from zero to 112. The different isolates were classified into 4 groups on the basis of sclerotial pattern i.e. peripheral, centre, scattered and ring in the middle. The isolates were classified into smooth, rough, smooth pitted and rough pitted on the basis of sclerotia texture.

Introduction

Soybean is called “Golden Gift” of the nature to mankind due to its various uses. Since time immemorial, soybean has served as milk, cheese, bread as well as oil for the people of China and East Asia; and the ancient literature of these countries called it as “gold from soil”. Off late, its versatility was recognized by the west, which called it “golden bean” or “miracle bean”. Due to its immense potential as food, fodder, feed, fuel and industrial production; soybean is said to have revolutionized the agricultural economy of west, particularly of United States. USA, Brazil, Argentina, China and India are the top five countries in the world with respect to area and production. In Chhattisgarh major soybean growing districts are Rajnandgaon, Durg, Mungeli, Bemetara and Kabirdham.

Soybean is mainly grown during Kharif season in sandy loam to clay loam soil in Chhattisgarh. Soybean plant is known to suffer from many diseases such as *Rhizoctonia* web blight, anthracnose, rust, *Cercospora* leaf spot, target spot, bud blight, yellow mosaic and some non-parasitic diseases due to excesses and deficiencies of trace elements (Sinclair, 1982). Among the fungal diseases *Rhizoctonia* web blight is one of the most important disease which attack the soybean plant towards maturity and directly affect on yield of soybean particularly in humid and warm part of the country and 35-60 percent yield losses have been estimated by Ray *et al.,* (2007). Web blight disease is becoming a new problem for soybean crop in Chhattisgarh and occurs in severe form in all soybean growing areas of Chhattisgarh. The knowledge about this disease is very meagre. Keeping in view the wide occurrence of disease and its destructive nature present investigation "Morphological, cultural and sclerotial variability among isolates of *Rhizoctonia solani* causal agent of web blight of soybean" has been undertaken.

Materials and Methods

The present investigation was carried out at the Research farm and laboratory of Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur.

Collection of diseased sample

An intensive survey was conducted during kharif 2016 in soybean growing districts of Chhattisgarh. The soybean leaves naturally infected with *Rhizoctonia* aerial blight symptoms were collected from the infected plants from farmers fields at different locations of Chhattisgarh (Rajnandgaon, Durg, Bemetra, Kabirdham and Raipur). Collected samples were brought to the laboratory for critical examination of the symptoms and identification and morpho-cultural variability studies. A total of 17 isolates of *R. solani* were obtained.

Morphological variability

Morphological characters like hyphal width, angle of hyphal branching and distance between two septa were examined for each isolate by using Olympus OSM Digital counter mounted on Olympus Vanox-S AHBS microscope.

Cultural variability

Isolates of *R. solani* were grown on fresh PDA medium plates at 27 ± 2^{o}C in incubator for studying colony characters such as colour of colony, colony texture,/appearance, radial growth.

Sclerotial variability of *R. solani* isolates

Time taken for sclerotial formation, colour, weight, number, sclerotial pattern, sclerotia location, texture of sclerotia and honey dew secretions in petriplate of each isolate were recorded at regular intervals after incubation at 27±2ºC in sterilized plates containing PDA medium. Munsell's colour chart was used to record the colour of the sclerotia. The sclerotial bodies placed on the sterilized glass slide and the key colour cards were kept side by side against white background under sunlight for comparison (Burpee *et al.*, 1991).

Results and Discussion

Morphological variability

Among the 17 isolates the lowest hyphal width was observed to be that of RS 10 (2.16 µm) and highest hyphal width was observed to be that of RS 14(4.38 µm) followed by RS 8 (4.34 µm), RS 11(4.27 µm), RS 12(4.17 µm),

RS 13 (4.11 µm), RS 3 (4.06 µm), RS 4 (4.05 µm), RS 2 (3.40 µm).They were statistically at par with each other. The observations were found to be in accordance with that of Kuiry *et al.*,(2014) who observed the hyphal width ranged from 2.25 to 13.08 µm. Among the 17 isolates the least angle of branching was observed to be that of RS 12(77.45˚) and the highest angle of branching was observed to be that of RS 5(95.743˚). Significant variation in distance between septa was also observed among the isolates (36.34 to 11.33 µm).

Isolate RS 4 was found significantly superior and formed higher distance between two septa (36.34 µm) followed by RS 7(34.00µm) which was at par with each other (Fig. 1).

Cultural variability

Based on the observation of colony colour the different isolates were assigned into three different groups i.e. pale white, light brown and ivory. Out of 17 isolates, 8 isolates were pale white in appearance. Five isolates were light brown in appearance. Four isolates were ivory in appearance. The results are in accordance with Lal and Kandhari (2009) who while studying variability of *R. solani* isolates found 6 isolates as light brown, five isolates were found yellowish brown, four isolates were whitish brown in colour, six isolates were dark brown and four isolates were very pale brown. Based on the observation of colony texture the different isolates were assigned into four different groups i.e. sparse, sparse fluffy, cottony and cottony fluffy.Out of 17 isolates, 6 isolates i.e. RS 7,RS 9, RS 10, RS 12, RS 14, RS 17 were sparse in appearance. Seven isolates i.e. RS 1,RS 2,RS 3, RS 5, RS 11, RS 13, RS 17 were sparse fluffy in appearance. Two isolates i.e. RS 4 and RS 15 were cottony in appearance. Two isolates i.e. RS 6 and RS 8 were cottony fluffy in appearance. The mycelial growth of the isolates varied from 21.6 mm (RS 5) to 54 mm (RS 15) after 24 hours. All the isolates except RS 13 (86.3 mm) showed full growth (90 mm) after 48 hours). Lal and Kandhari (2009) reported that ten isolates covered the whole plate (90mm) in 48hours.

Sclerotial variability

The number of sclerotia formed ranged from zero to 112.The maximum number of sclerotia formed in RS 14 (112) followed by RS 8(110) which was statistically at par with each other. The number of sclerotia formed in five isolates were very high (>100). The number of sclerotia formed in six isolates was high (51-100). The number of sclerotia formed in RS 11 and RS 17 were moderate (26-50).

The number of sclerotia formed in RS 7 was low (1-25). The different isolates were classified into 4 groups on the basis of sclerotial pattern i.e. peripheral, centre, scattered and ring in the middle. It was seen that in eight isolates sclerotial arrangement was on surface i.e. aerial sclerotia was absent. Both aerial and surface sclerotia were observed in case of 8 isolates. Lal and Kandhari (2009) reported that in 15 isolates sclerotia were formed on surface of mycelium and 9 were both aerial and surface.

The different isolates were classified into 4 categories i.e. smooth, rough, smooth pitted and rough pitted on the basis of sclerotia texture. The majority of isolates were found to be rough pitted in nature. Three isolates were smooth pitted in nature. One isolate i.e. RS 10 was found to be rough in nature. Four isolates were found to be smooth in nature. The different isolates were grouped into 4 categories on the basis of sclerotia colour i.e. light brown, brown, dark brown and grey. Four isolates were light brown in colour. Four isolates were brown in colour. Seven isolates were found to be dark brown in colour i.e. One isolate was observed to be grey in colour (Fig. 2).

Table.1 Cultural variability among different isolates

Isolate	Colony colour	Colony texture/appearance	Mycelial growth(mm/hours)		
			24	48	72
RS 1	Pale white	Sparse fluffy	24.96	90.00	-
RS 2	Pale white	Sparse fluffy	27.60	90.00	-
RS 3	Light brown	Sparse fluffy	26.88	90.00	-
RS 4	Light brown	Cottony	31.20	90.00	-
RS 5	Ivory	Sparse fluffy	21.60	90.00	-
RS 6	Ivory	Cottony fluffy	40.38	90.00	-
RS 7	Pale white	Sparse	27.80	90.00	-
RS 8	Pale white	Cottony fluffy	43.44	90.00	-
RS 9	Pale white	Sparse	34.80	90.00	-
RS 10	Light brown	Sparse	37.20	90.00	-
RS 11	Light brown	Sparse fluffy	34.80	90.00	-
RS 12	Light brown	Sparse	37.20	90.00	-
RS 13	Pale white	Sparse fluffy	28.80	86.30	90.00
RS 14	Pale white	Sparse	26.60	90.00	-
RS15	Ivory	Cottony	54.00	90.00	-
RS 16	Ivory	Sparse fluffy	51.12	90.00	-
RS 17	Pale white	Sparse	34.56	90.00	-

Table.2 Sclerotial variability among different isolates

Isolate	Sclerotia initiation	Weight (mg)	Sclerotia (No/plt)	Sclerotial pattern	Arrangement surface/aerial	Sclerotia texture	Sclerotia colour	Honeydew Formation (hrs)
RS 1	48	21.50	85	Peripheral	Surface	Rough pitted	Dark brown	No
RS 2	72	10.46	82	Centre	Both	Smooth pitted	Light brown	No
RS 3	72	26.34	105	Peripheral	Surface	Rough pitted	Brown	No
RS 4	72	23.96	73	Peripheral	Both	Rough pitted	Dark brown	No
RS 5	72	25.25	108	Scattered	Surface	Rough pitted	Dark brown	No
RS 6	48	22.14	102	Scattered	Both	Smooth pitted	Dark brown	No
RS 7	48	25.60	14	Scattered	Both	Rough pitted	Brown	No
RS 8	48	19.61	110	Scattered	Both	Smooth	Light brown	No
RS 9	48	22.40	91	Peripheral	Both	Smooth	Brown	No
RS 10	48	15.18	63	Peripheral	Both	Rough	Brown	No
RS 11	48	21.39	38	Scattered	Both	Smooth	Light brown	No
RS 12	-	-	-	-	-	-	-	-
RS 13	72	24.73	41	Centre	Surface	Smooth	Dark brown	No
RS 14	72	26.94	112	Peripheral	Surface	Rough pitted	Dark brown	No
RS 15	72	26.99	101	Peripheral	Surface	Rough pitted	Dark brown	No
RS 16	72	25.37	66	Ring in middle	Surface	Rough pitted	Light brown	No
RS 17	72	27.78	27	Centre	Surface	Smooth pitted	Grey	Yes
SE (m) ±		0.573	0.950					
CD(5%)		1.723	2.741					

Fig.1 Morphological variability among different isolates

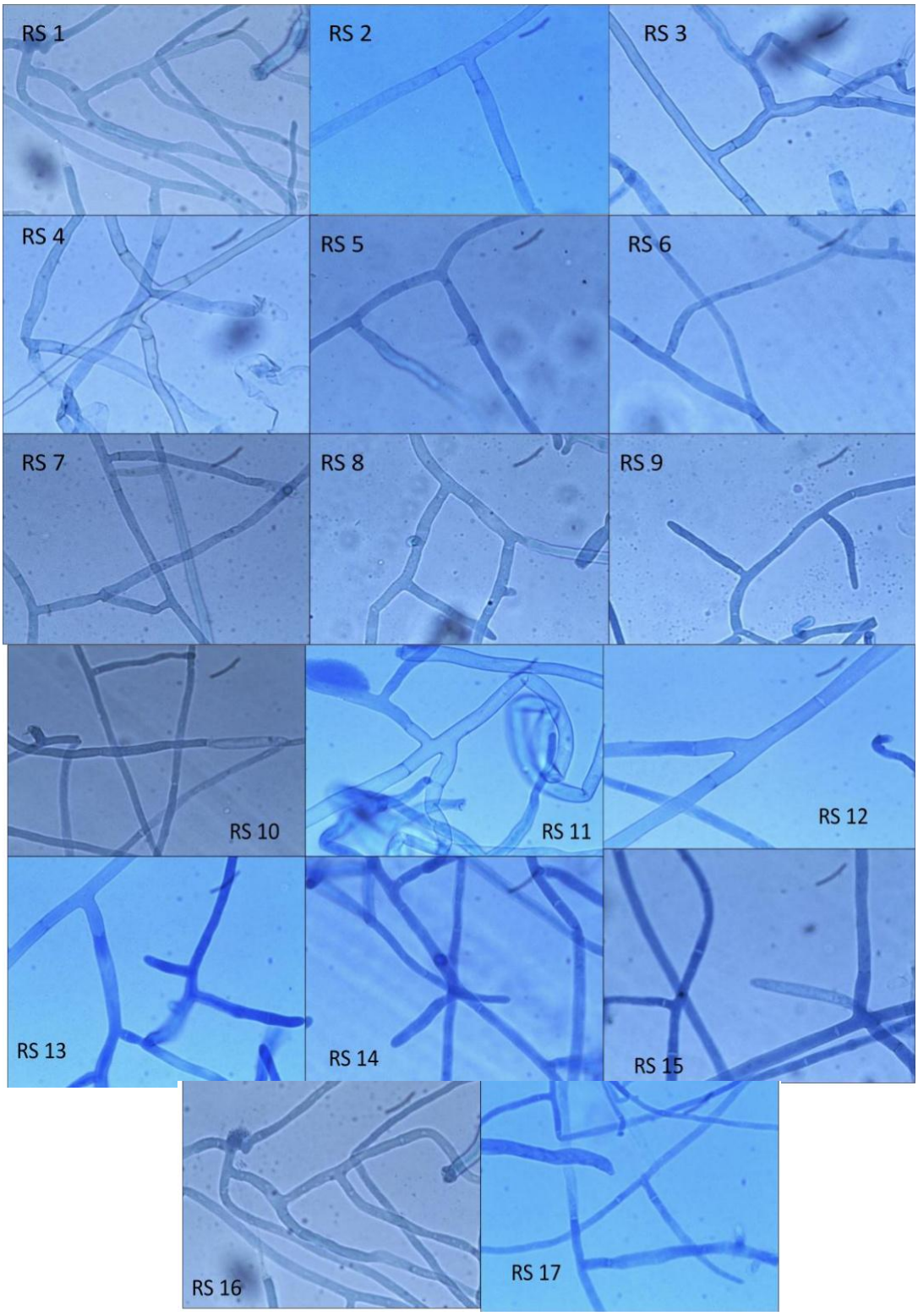
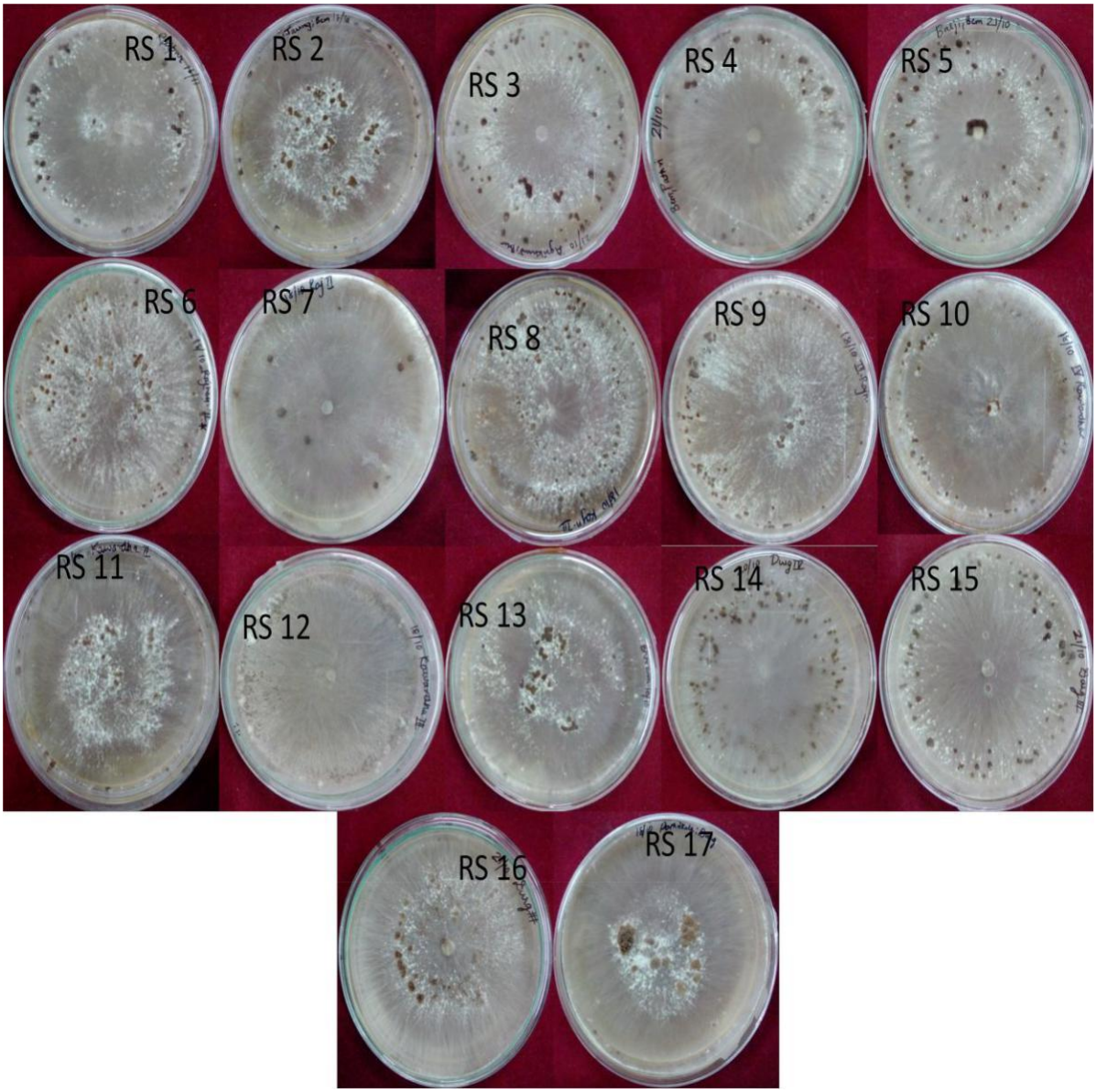


Fig.2 Different isolates of *R. solani* collected from survey



The sclerotial weight ranged from 10.46 mg to 27.78mg. Maximum sclerotial weight was recorded in RS 17(27.78 mg). The minimum sclerotial weight was recorded in RS 2(10.46 mg). In case of seven isolates sclerotia initiation was found to occur after 48 hours whereas in case of nine isolates sclerotia initiation was found to occur after 72 hours. Honeydew formation was observed in only 1 isolate i.e. RS 17 while in the other isolates honeydew formation was not seen.

It was observed that all the isolates covered the entire plate (90mm) after 48 hours except RS 13 (86.30 mm). Eight isolates were found pale white, five isolates light brown and four isolates were ivory in colony colour. Seven isolates were sparse fluffy, six isolates were sparse, two isolates were cottony and two were cottony fluffy in colony texture. The highest hyphal width (4.38µm) was recorded in case of RS 14 which was followed by RS 8.The highest angle of branching was

observed for RS 5(95.743˚) and least for RS 12(77.45˚).The highest distance between two septa was recorded in case of RS 4(36.34µm) and least for RS 14(11.33µm). Seven isolates showed sclerotia initiation after 48 hours while nine isolates showed sclerotia initiation after 72 hours. The highest sclerotial weight (27.87 mg) was recorded for RS 17. The highest number of sclerotia (112) was observed in RS 14, followed by RS 8(110). Eight isolates were found to be rough pitted, three were smooth pitted, four were smooth and one was rough in terms of sclerotial texture. Seven isolates were dark brown, four isolates were light brown, four isolates were brown and one was grey in terms of sclerotia colour. The sclerotial arrangement pattern of seven, three, five and one isolate was peripheral, central, scattered and ring in middle respectively. Honeydew formation was not observed in all the isolates except RS 17.

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