Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 1326-1329
Received: 25-09-2019
Accepted: 27-10-2019

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Cultural and pathogenic variability of *Rhizoctonia solani* causing root rot of soybean in Madhya Pradesh

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Abstract
Soybean [*Glycine max* (L.) Merr] is one of the most important legumes, rich in protein and oil, which can be used in agriculture and oil extraction industry. *Rhizoctonia solani*, where causing foot and root rots of young soybean plants which characterized by the browning of the vascular tissue of roots and stems.The present investigation were *Rhizoctonia solani* isolates collected from different Places viz Seoni, Katni, Balaghat, Bhopal, Mandala, Jabalpur, Sehora and Indore of Madhya Pradesh.These ten isolates were studied their Cultural and Pathogenic variability . The cultural variability were observed that different colony colour and average colony diameter were whitish grey, white, brown grey colour but most of isolates whitish grey-I_1, I_2, I_5, I_6, I_8 and maximum colony diameter is I_4, I_1, I_9 Followed By I_6, I_{10}.The Pathogenic Variability Observations were Maximum disease incidence recorded at pre- emergence stage was I_{10} (20.00) and minimum disease incidence with I_1 (6.67) followed by I_3, I_7, I_9. Maximum disease incidence was recorded on post emergence stages with I_9 (69.55) and minimum disease incidence recorded with I_5 (6.67). However isolate I_9 was found more pathogenic as it exhibited highest (76.22) mortality as compared to other isolates.

Keywords: Soybean, cultural and pathogenic variability, *Rhizoctonia solani* isolates

Introduction
Soybean [*Glycine max (L.)* Merill] is the major oilseed crop in the world and is cultivated on an area of 108.83 Lakh ha with a total production of 114.90 Lakh MT. The crop is grown in a wide range of agro climatic zones. The low yield of soybean in India can be attributed to legions of biotic and abiotic constraints. Among biotic factors, diseases are the most dominant. Soybean crop can be affected by more than 100 pathogens (Sinclair and Shurlleff, 1975) [20]. Among all these pathogens, *Rhizoctonia spp.* is the most destructive pathogen for this crop causing heavy yield losses every year. The root rot pathogenic fungi are major threat for this crop as these fungi attack on the root of the plant and damage the crop. *Fusarium* spp., *Rhizoctonia solani* and *Pythium* spp. are considered as major soybean seedling pathogens, which contribute to stand reduction (Inam-Ul-Haq *et al.,* 2012) [9]. Root rot has become an important disease of soybean caused by *Rhizoctonia solani* in recent past. Hence, a detailed and systematic study is required to manage this important disease.There are reports in other parts of the world that populations of *Rhizoctonia solani* showed significant variations morphologically, physiologically, pathogenically and genetically. These variations aid the pathogen to adapt and survive in diverse environments. A thorough knowledge of pathogenic variability of *Rhizoctonia solani* is essential to design disease management strategies for different agro climatic zones of the country by breeding resistant cultivars. Hence, we presently investigated morphological, cultural and pathogenic characteristics among 10 isolates of *Rhizoctonia solani* infecting soybean, collected from ten different major soybean growing areas of Madhya Pradesh in India.

Material and Methods
Test pathogen
Rhizoctonia solani, the causal organism of soybean root rot isolated from diseased plant was used as test pathogen.

Collection
During the month of August to September 2017 survey was made. Sample plants showing the root rot symptoms and infected plants showing yellow leaves upward from the base.

Circular patches of stunted plants were also observed from different places (Seoni, Katni, Balaghat, Bhopal, Mandala, Jabalpur, Sehora and Indore).

Isolation

The roots having typical symptoms were used for isolation. The diseased plants of soybean were collected and causal organisms were isolated from affected roots of soybean plants. The diseased roots were first washed in tap water to remove dust particles and were cut into small pieces (1-cm) with a sterilized scalpel. The cut pieces were surface sterilized with 0.1 per cent solution of sodium hypochlorite under aseptic conditions for 30 seconds. These pieces were transferred to Potato Dextrose Agar (PDA) medium in 90 mm Petri dishes aseptically. The Petridishes were then placed in BOD incubator at 25±1ºC temperature for 7 days.

Purification and identification of associated pathogen

The culture of *Rhizoctonia solani* was purified by hyphal tip method. To obtain the sparse growth, the test pathogens were inoculated on sterilized water agar in Petriplates from the original culture petriplate. After two days the growth of the fungus was carefully examined under low power (10 xs) of microscope from the reverse side of Petriplate. Agar disc (2mm) corresponding to the marked area was cut with a sterilized cork borer and transferred aseptically on PDA in Petriplate and incubated at room temperature 25±1ºC. Culture of *R. solani* was identified on the basis of characters illustrated by Ceresini (1999) [5].

Cultural variability

Isolates of *Rhizoctonia solani* were grown on fresh PDA medium plates at 25+1ºC. Colony characters such as Growth rate, Colour, diameter of the colony were studied until the mycelium reached the periphery of the plate.

Colony Texture

Colony growth of each isolate was recorded as aerial fluffy or submerged or running on 7th day old culture. Based on mycelial growth and appearance the isolates were designated to different groups.

Radial growth

For measuring the radial growth rate, all the ten isolates of *Rhizoctonia solani* were inoculated at the centre of 90mm PDA in different plates and maintained four replication of each isolate. Inoculum was in the form of 5mm mycelium disc. The plates were incubated at 25±1ºC and the radial growth was measured after 1day, 2 days, 3 days, 4 days and 5 days of incubation period.

Pathogenic variability by Soil infestation

The inoculum was thoroughly mixed in sterilized sand + soil (1:1) @ 100g/ 2 kg soil. The sterilized soil was mixed with inoculum multiplied on corn meal sand medium. Soybean seeds (JG 95-60) were surface sterilized with 0.1% mercuric chloride for one minute and washed properly. Five seeds were placed in one earthen pot and three replications were maintained. These pots were kept in a net house. Proper isolation was maintained to avoid other pathogens. Observations on germination, pre and post emergence mortality were recorded. No soil treatment with test fungus served as control.

Results and Discussions

The present investigation was undertaken to find out the variability in different isolates of *Rhizoctonia solani* causing root rot of soybean.

Collection and isolation of *Rhizoctonia solani* from root of soybean

Soybean plants showing symptoms of root rot were collected from soybean field of different districts of M.P. Samples were brought into the Department of Plant Pathology for isolation and further studies (Table 1).

Table 1: *Rhizoctonia solani* isolates collected from different districts of Madhya Pradesh

Sr. No.	Place	Indicated symbol(I)
1	Balaghat	Isolate-I_1
2	Mandala	Isolate -I_2
3	Indore	Isolate -I_3
4	Bhopal	Isolate -I_4
5	Jabalpur	Isolate -I_5
6	Sehora	Isolate -I_6
7	Jabalpur	Isolate -I_7
8	Katni	Isolate -I_8
9	Seoni	Isolate -I_9
10	Indore	Isolate -I_{10}

Table 2: Colony characters (colour, type, edge) of ten isolates of *Rhizoctonia solani* grown on PDA at 25±2ºC

Isolates. No.	Colony colour	Growth pattern	Colony edge
I_1	Whitish grey	fluffy	Smooth
I_2	White with grey ring	fluffy	Undulating
I_3	Whitish	fluffy	Smooth
I_4	Grey	fluffy	Wavy
I_5	Whitish grey	submerged	Smooth
I_6	Whitish grey	fluffy	Smooth
I_7	Grey	cottony	Smooth
I_8	Grey with white ring	Slightly cottony	Smooth
I_9	White	Fluffy	Undulating
I_{10}	Brown	Slightly fluffy	Smooth

Four types of colony colour were observed with the 10 isolates of *Rhizoctonia solani*, of which whitish grey was recorded with I_1, I_2, I_5, I_6 and I_8 whereas white colour was recorded with I_3 and I_9, brown colour recorded with I_{10} and grey colour recorded with I_7 and I_4. Mycelia growth pattern was also observed with 10 isolates of *Rhizoctonia solani*, of which fluffy mycelial growth pattern was recorded with I_1, I_2, I_3, I_4, I_6 and I_9, whereas, submerged I_5, cottony I_7, slightly cottony I_8 and I_{10} exhibited brown colour. Colony edge was also observed, smooth, wavy, undulating.the smooth colonies were I_1, I_3, I_5, I_6,I_7, I_8 and I_{10} waxy colony I_4 and undulating colony I_2, I_9.Similar morphological characters were reported

by Exner (1953) [6], Richter and Schneider (1953) [17], Van Eijanathan (1961) [22], Meyer (1965) [13], Papavizas (1965) [16], Sherwood, (1970) [19], Bollen (1972) [4], Kuninaga *et al.* (1978) [11], Matsuyama *et al.* (1978) [12] Naiki and Ui (1978) [15] and Adams and Butler (1979) [2].

Table 3: Average colony growth of ten isolates of *Rhizoctonia solani* on potato dextrose agar medium after different incubation period at 25±2ºC

Isolates	Average colony growth of *Rhizoctonia solani* (mm) after		
	48hrs	72hrs	96hrs
I_1	53.33	88.33	90.00
I_2	32.50	55.00	75.00
I_3	25.00	52.50	82.50
I_4	63.33	90.00	90.00
I_5	43.33	61.66	71.66
I_6	47.50	78.33	88.33
I_7	35.00	60.83	89.16
I_8	22.00	19.16	29.16
I_9	40.00	72.50	90.00
I_{10}	44.16	74.16	87.50
SE(m)±	1.367	0.74	1.46
CD5%	4.06	7.42	4.35

Growth rate of ten isolates of *Rhizoctonia solani* were recorded on PDA at different incubation period and were incubated at 25±2ºC.From the data recorded in Table 3 that all isolates of *Rhizoctonia solani* varied significantly in their growth at different incubation period. Mycelial growth increased with increase in incubation period. Maximum growth of 90.00 mm. was recorded with isolate I_4 after 72hrs of incubation followed by 88.3, 78.3, 74.16 and 72.50, with isolate I_1, I_6, I_{10} and I_9 respectively. Similarly after 96hrs of incubation period 90 mm. Colony growths exhibited with I_1, I_4 and I_9 Isolates, while minimum of 29.16 mm growth recorded with I_8. Overall isolate I_4 exhibited maximum growth at all incubation period. Similar results have been reported by Basu and Gupta (1992) [3], Guleria *et al.* (2007) [8], Goswami *et al*. (2008) [7], Khodayari *et al*. (2009) [10], Sharma *et al.* (2013) [13] and Mishra *et al.* (2014) [14].

Pathogenic variability

To record pathogenic variability, studies were conducted in polyhouse by soil infestation of *Rhizoctonia solani* on soybean variety JS 95-60. Where pre emergence mortality and post emergence mortality were recorded and presented in Table 4.

Table 4: Pathogenic variability of different isolates of *Rhizoctonia solani* by soil infestation method.

Isolates	Germination %	Mortality %		
		Pre emergence	Post emergence	Total mortality
I_1	93.33	6.67	21.67	28.34
I_2	86.67	13.33	46.66	59.99
I_3	93.33	6.67	30.00	36.67
I_4	100.00	0.00	20.00	20.00
I_5	66.00	0.00	6.67	6.670
I_6	86.67	13.33	31.67	47
I_7	93.33	6.67	28.33	35
I_8	86.67	13.33	36.67	50
I_9	66.67	6.67	69.55	76.22
I_{10}	80.00	20.00	50.00	70
Control	86.67	0.0	0.0	0.0
SE(m)±	1.15	3.08	2.63	
CD5%	3.40	1.04	7.77	

The data presented in Table 4 indicate that pre emergence mortality varied from 6.67 to 20.00. Maximum disease incidence recorded at pre- emergence stage was I_{10} (20.00) and minimum disease incidence with I_1 (6.67) followed by I_3, I_7, I_9. Whereas post emergence mortality varied from 6.67 to 69.55. Maximum disease incidence was recorded on post emergence stages with I_9 (69.55) and minimum disease incidence recorded with I_5 (6.67). However isolate I_9 was found more pathogenic as it exhibited highest (76.22) mortality as compared to other isolates. Present findings are similar to the results, variability and virulence among the different isolates of *Rhizoctonia solani* reported by Tiwari and Khare (1998) [21] and Abdulnabi *et al*. (2017) [1].

References

1. Abdulnabi AA, Matrood and Azher HA. Inhibition activity of Mycorrhizal fungi *Glomus mosseae* and *G. intradicas* with *Tricoderma harzianum* against *Rhizoctonia solani* in Okra plant *Abelmoschus esculentus* (L.). Basrah Journal of Agricultural Sciences. 2017; 30(2):72-82.
2. Adams GC and Butler EE. Serological relationships among anastomosis groups of *Rhizoctonia solani*. Phytopathology, 1979; 69:629-633.
3. Basu A, Gupta PKS. Cultural and pathogenic variation in rice isolates of *Rhizoctonia solani* Kuhn. Beitrage zur Tropischen Landwirtschaft and Veterinarmedizin, 1992; 30(3):291-297.
4. Bollen GJ. A comparison of the in vitro antifungal spectra of thiophanates and benomy. Netherland Journal of Plant Pathology. 1972; 78:55-64.
5. Ceresini P. 1999. https:// projects. ncsu.edu/cal s/course/pp728/ Rhizoctonia/ Rhizoctonia.html.
6. Exner B. Comparative studies of four *Rhizoctonia* occurring in Louisiana. Mycologia. 1953; 45:698-719.
7. Goswami BK, Bhuiyan KA, Mian IH. Cultural and morphological variation in the isolates of *Rhizoctonia solani*. Bangladesh Journal of Plant Pathology 2008; 24(½):63-68.
8. Guleria S, Aggarwal R, Thind TS, Sharma TR. Morphological and pathological variability in rice isolates of *Rhizoctonia solani* and molecular analysis of their genetic variability. Journal of Phytopathology, 2007; 155:654-661.
9. Inam-Ul-Haq, Sajid MM, Hafiz R, Ali M, Zahid. Tahir MJ. Incidence of root rot diseases of soybean in Multan Pakistan and its management by the use of plant growth

promoting Rhizobacteria. Pakistan J Bot. 2012; 44(6):2077-2080.
10. Khodayari M, Safaie N, Shamsbakhsh M. Genetic diversity of Iranian AG1-IA isolates of *Rhizoctonia solani*, the cause of rice sheath blight, using morphological and molecular markers. Journal of Phytopathology. 2009; 157(11/12):708-714.
11. Kuninaga S, Yokosawa R, Ogoshi A. Anastomosis grouping of *Rhizoctonia solani* Kuhn., isolated from non-cultivated soil. Annual Phytopathological Society of Japan 1978; 44:591-598.
12. Matsuyama N, Moromizato Z, Ogoshi A and Wakimoti S. Grouping *Rhizoctonia solani* Kuhn with non-specific esterase zymogram. Annals of the Phytopathological Society of Japan. 1978; 44: 652-658.
13. Meyer RW. Heterokaryosis and Nuclear Phenomenon in *Rhizoctonia*. Ph.D. Thesis, University of California, Berkeley. 1965, 118.
14. Mishra PK, Gogoi R, Singh PK, Rai SN, Singode A, Kumar A *et al.* Morpho-cultural and pathogenic variability in *Rhizoctonia solani* isolates from rice, maize, and green gram. Indian Phytopath, 2014; 67(2):147-154.
15. Naiki T, Ui T. Ecological and morphological characteristics of sclerotia of *Rhizoctonia solani* kuhn produced in soil. Soil Biology and Biochemistry, 1978; 10:471-478.
16. Papavizas GC. Comparative studies of single baridiospore isolates of *Pellicularia filamentosa* and P. Praticola. Mycologia. 1965; 57:91-103.
17. Richter R. Schneider R. Unterruchungen Zear Morphologische and Biologischen Differen-ziercing Von *Rhizoctonia solani*. Phytopathology Zeitschrift. 1953; 20:167-226.
18. Sharma L, Goswami S, Nagrale DT. Culture and physiological variability in *Rhizoctonia solani*, responsible for foliar and lesions on aerial part of soybean. Journal of Applied and Natural Science. 2013; 5(1):41-46.
19. Sherwood RT. Physiology of *Rhizoctonia solani*. In Parameter, I. R. Jr. (ed.), *Rhizoctonia solani* biology and pathology. London: University of California Press. 1970, 69-92.
20. Sinclair JB, Shurlleff MC. Compendium of soybean diseases. Amer. Phytopath. Soc. Inc. Minnesota, USA: 1975, 8-10.
21. Tiwari A, Khare MN. Variability among isolates of *Rhizoctonia solani* infecting mungbean. Indian Phytopath 1998; 51(4):334-337.
22. Van Eijanthan CLM. Annual report of the Department of Agricultural Research. Federation of Nigeria for the year 1961; 63:1959-60.