

Original Research Article

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Variability among Isolates of *Rhizoctonia solani* Inciting Web Blight of Mungbean

Jai Singh¹ and Ashish Kumar^{2*}

¹JNKVV, Krishi Vigyan Kendra, Sidhi-486661 (M.P.), India

²Plant Pathology, JNKVV, College of Agriculture, Jabalpur (M.P.)-482 004, India

*Corresponding author

ABSTRACT

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Web blight of mungbean is one of the important diseases of mungbean (*Vigna radiata* L.). Morphological and pathogenic variability of 48 isolates of *R. solani* from 5 different districts of Uttar Pradesh were studied. Majority of the isolates produced pale brown mycelium with fluffy growth and were categorized into 3 groups on the basis of their growth rate including 11 fast growing, 25 moderately growing and 12 slow growing isolates of *R. solani*. Sclerotia formed in different isolates were highly variable in number as well as size and it was observed that most of the isolates produced sclerotia of 1-2 mm diameter. Most of the sclerotia were scattered followed by peripheral in Petri plates. The isolates were also variable in respect of their virulence and grouped into three categories such as high (19), moderate (26) and less virulent (3) isolates.

Introduction

Web blight of Mungbean (*Vigna radiata* L.) caused by *Rhizoctonia solani* Kühn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is an economically important disease which reduces 33 to 40 per cent grain yield and 28.6 per cent in 1000 grain weight at different level of disease severity and in different varieties of mungbean (Singh *et al.*, 2012 and Gupta *et al.*, 2010) due to destruction of leaves or seed blemishes that reduces its value.

The symptoms of web blight of mungbean caused by *R. solani* can be observed as leaf and pod spots, leaf blights, defoliation, stem and petiole lesions, cob-web like mycelium

and produces abundant microsclerotia on diseased plant tissue during growing season (Singh *et al.*, 2013). The disease is spread by mycelial bridges between plants, rain splashed sclerotia, infested soil debris (Galvez *et al.*, 1989) and air borne basidiospores (Cardenas-Alonso, 1989). Use of resistant variety is the most economical management strategy. However, available commercial mungbean cultivars differ somewhat in susceptibility but no highly resistant commercial cultivars have been envisaged.

Other management options including cultural and use of costly fungicides, are neither highly effective nor economic. Further, the judicious use of pesticides has led to hazardous effect on soil and environment also.

The pathogen *R. solani* is cosmopolitan in nature with a wide host range and attacks large number of crop plants and weeds. It is claimed that there is hardly any plant species which cannot be infected by the pathogen (Ogoshi, 1987). As a plant pathogen *R. solani* regarded as unspecialized fungus and composed of a 14 complex assemblage of anastomosis groups (AGs) including AG-1 to AG-13 and AG BI. These AGs are genetically distinct non-mating populations and have a wide variation in their morphological, cultural, pathological and physiological characteristics (Kuninga *et al.*, 1997). Among these AGs, isolates of AG 1 have been divided into three sub groups; AG 1-IA, AG 1-IB and AG 1-IC (Sneh *et al.*, 1991) and web blight of mungbean in India is caused by AG 1-IB (Singh, 2006). The web blight isolates of *R. solani* from mungbean and soybean are varying in their cultural, morphological appearance, pathology, anastomosis and physiology. To develop resistant variety in any successful breeding programme, it is very essential to know the existence of variability in the population of *R. solani* AG 1-IB causing web blight of mungbean. Also information on cultural, morphological and pathogenic variability can enlighten in selection of virulent strains for identification of host resistance. Even though variability in mungbean isolates of *R. solani* had been reported by some workers in past, it needs restudy as the behavior and virulence of the isolates may change through natural mutation and gene shift over time. The degree of disease severity on host plants also depends on the fungal pathotype.

Therefore, information on variability among different isolates of *R. solani* is an important aspect of research for developing eco-friendly management of web blight of mungbean. Hence, present investigation was aimed to study the cultural, morphological and pathological variability in isolates of *R. solani* causing web blight of mungbean.

Materials and Methods

Collection and establishment of isolates of *R. solani*

An extensive roving survey was conducted in mungbean growing areas of five districts Eastern Uttar Pradesh. Most of the mungbean fields suffered severely with web blight disease. The mungbean leaves showing typical web blight symptoms were collected in polythene bags and details of samples and place of collection were recorded. The pathogen was isolated from the infected leaves showing typical web blight symptoms by tissue segment method on potato dextrose agar medium (PDA). Small bits measuring about 3 mm size were cut off from the leaves showing lesions in such a way that it contained both infected and healthy portions and these bits were surface sterilized in 0.1 per cent mercuric chloride (HgCl₂) for 30 seconds followed by three washings in sterile distilled water. The bits were further transferred to sterile discs of blotting paper. The dried bits were subsequently transferred to potato dextrose agar (PDA) medium under aseptic conditions. The petriplates were incubated at 28±2⁰C for seven days for the growth of the fungus. The developed fungal colonies were purified by hyphal tip cut isolation method (Rangaswami and Mahadevan, 2004). The pathogen was identified as and maintained on PDA slants under controlled temperature.

Cultural characterization

For cultural characterization, all the isolates of *R. solani* were inoculated in four replications at the centre of 90 mm PDA plates. Inoculum was in the form of 5 mm mycelial discs taken from margin of colonies grown on PDA plates. The plates were incubated at 25°C and the radial growth was measured (in mm) 7 days post inoculation. Four replications were maintained for each isolates. Colony diameter

(in mm), mycelial abundance, zonation, colour and pattern of sclerotia formation, number of sclerotia per plate, size and type of sclerotia were recorded seven day after inoculation. The colony diameter of each isolate was measured at the interval of 24h, 48h, 72h and 96h to calculate the growth rate (mm/hour). Observation on mycelia abundance and position of sclerotia formation were recorded on the basis of key given by Burpee *et al.*, (1980) and accordingly, mycelial abundance were grouped in three categories: slight (aerial mycelium does not obscure surface mycelium); moderate (aerial mycelium obscures surface mycelium but does not touch the cover of petriplates) and abundant (aerial mycelium obscures surface mycelium and touches the cover of petriplates). Location of sclerotia formation was categorized into (i) aerial (sclerotia formation within or on the aerial mycelium), (ii) surface (sclerotia formation on the surface mycelium) and (iii) embedded (sclerotia formation within the substrate).

Morphological and pathological characterization

The morphological diversity of isolates of *R. solani* was determined by studying various phenotypic characters as described by Kuninaga *et al.*, (1978). Hyphal width was recorded with the help of micrometer which was previously calibrated with stage micrometer. About hundred measurements were taken for each isolate. For sclerotial diameter, sclerotia collected from 20 days old culture of different isolates were measured separately with the help of micrometer and simple ruler.

Virulence of different isolates of *R. solani* on mungbean was determined according to Tiwari and Khare (1998). One month old trifoliolate leaves of mungbean (SML-3) plant, were inoculated with 6 mm diameter mycelial

disc of each isolate taken from 3 day old culture after thoroughly washing with sterilized distilled water. Host petioles were dipped in 250 ml sterilized water in a conical flask. The flasks were kept under Belljar's lined with moist blotter. To maintain relative humidity of about 100 percent, blotter paper was moistened with sterilized water twice in a day. Suitable control was maintained where only PDA disc was used. The belljars were kept at room temperature ranging from 18 to 32°C (night and day) for five days. After five days, each leaflet was evaluated for disease symptoms based a 1-5 rating scale (Muyolo, 1993) where, 1 = no symptoms; 2 = 1-25% leaf area blighted; 3 = 26-30% leaf area blighted; 4 = 51-75% leaf area blighted and 5 = 76-100% leaf area blighted. On the basis of degree of virulence isolates were grouped as less virulent (LV) caused = 0-50% disease severity, moderately virulent 50-80% disease severity and highly virulent group causing above 80% disease severity on trifoliolate of highly susceptible germplasm of mungbean.

Results and Discussion

Cultural and morphological variability

A field survey was conducted in different locations of Uttar Pradesh which included different blocks in different districts. In total, 5 districts of Uttar Pradesh including Azamgarh, Bhadohi, Jaunpur, Ghazipur and Varanasi, were surveyed for collection of web blight infected mung bean samples.

Among these 5 districts, web blight infected mung bean samples were collected from 48 locations. Disease samples were collected from 10 locations of each district except Jaunpur where 8 locations yielded in web blight infected samples of mung bean. List of different locations along with district and coding of *R. solani* isolates has been given in table 1.

All the isolates of *R. solani* exhibited variability in their growth rate (millimeter/hour) on PDA at $28\pm 1^{\circ}\text{C}$. The isolates were classified into three groups of fast ($>1.25\text{mm/hr}$. growth rate), moderate (1.0 to 1.25mm/hr . growth rate) and slow growing ($<1.0\text{mm/hr}$. growth rate). The growth rate of different isolates ranged from 0.73mm (B_8) to 1.25mm .

In total, 11 fast growing, 25 moderately growing and 12 slow growing isolates were observed. The compound microscopic studies revealed that all the forty eight web blight isolates of *R. solani* AG-1IB in present study characteristically having hyphal branching at right angle, construction at the point of the mycelium, presence of the septum near the branching junction and multinucleate hyphal cell which is of immense taxonomical importance (Sneh *et al.*, 1991).

The hyphal diameter of different isolates ranged from $5.13\ \mu\text{m}$ (B_1) to $10.21\ \mu\text{m}$ (G_6). In total, 34 isolates were having thick hyphae and more than $7.00\ \mu\text{m}$ hyphal diameter was recorded.

The colony characteristics with respect to colony colour revealed that maximum number of colonies was observed with light (13 isolates) and dark shade (15 isolates) of pale brown. However, colonies also depicted light grey (7 isolates), light yellow brown (5 isolates), pinkish white (1 isolate) and yellowish white (7 isolates).

This shows the dominance of pale brown colour of *R. solani* in PDA medium. Further, among 48 isolates of *R. solani*, only 18 isolates showed zonation and 30 isolates lack any kind of zonation in culture. The detail of different cultural characters including growth rate, hyphal diameter, colony colour and zonation of different isolates of *R. solani* has been given in table 2.

Sclerotial variability and virulence

Sclerotia are the resting structures of *R. solani* and sclerotial diversity of 48 isolates of *R. solani* was determined by studying of various characteristic of sclerotia like colour, formation pattern, duration of sclerotia initiation(hours), their number and size. It was observed that earliest sclerotia formation started from 72 hours and maximum duration for sclerotia initiation took 102 hours. Among 48 isolates, 11 isolates took 72 hours, 25 isolates took 84 hours, 9 isolates took 96 hours and 3 isolates took 102 hours for sclerotial initiation. Based on colour of sclerotia, isolates were categorized in to six groups including light reddish brown (one isolate), reddish brown (ten isolates), medium brown (six isolates), light brown (ten isolates) and dark brown (twenty one isolates). This shows the maximum occurrence of dark brown colour sclerotia in cultures of *R. solani*. Among different cultures of *R. solani*, sclerotia were formed mainly throughout the plate in irregular manner (21 isolates).

However, in 18 isolates sclerotia were formed on periphery. A set of six isolates showed formation of sclerotia in concentric rings. However, in 3 isolates sclerotia formation took place near inoculation point only. Sclerotia were mainly formed on surface of the culture (32 isolates). However aerial and partially embedded sclerotia were recorded in 5 and 3 isolates respectively. Average number of sclerotia produced by individual isolate varied from 40.66 to 243 per plate and maximum number of sclerotia/plate was observed in isolates A_4 whereas minimum number (40.66) was observed in isolate V_8 . Size of sclerotia of all forty eight isolates also varied in diameter and it was observed that most of the isolates produced sclerotia of 1-2 mm diameter. However, only five isolates produced large sclerotia of more than 2.0 mm diameter.

Table.1 Source of isolates of *R. solani* with their codes

S. No.	District	Location	Name of isolates
1.	Azamgarh	Tada	A ₁
2.		Rani sarai	A ₂
3.		Jiwali	A ₃
4.		Mauhari	A ₄
5.		Bhilihali	A ₅
6.		Chandesher	A ₆
7.		Budanpur	A ₇
8.		Tarawa	A ₈
9.		Bakesh	A ₉
10.		Devagoan	A ₁₀
11.	Bhadohi	Vikrampur	B ₁
12.		Aurai	B ₂
13.		Suriyava	B ₃
14.		Nawada	B ₄
15.		Maharajpur	B ₅
16.		Kandhiya	B ₆
17.		Khamaria	B ₇
18.		Bhadohi	B ₈
19.	Jaunpur	Saidabad	J ₁
20.		Pali	J ₂
21.		Sikarara	J ₃
22.		Ram dyalganj	J ₄
23.		Palahamau	J ₅
24.		Chandwack	J ₆
25.		Sirkoni	J ₇
26.		Madhopatti	J ₈
27.		Patrahi	J ₉
28.		Birbhanpur	J ₁₀
29.	Ghazipur	Narayanpur	G ₁
30.		Deva	G ₂
31.		Badhupur	G ₃
32.		Khalispur	G ₄
33.		Mudwal	G ₅
34.		Saidpur	G ₆
35.		Nandganj	G ₇
36.		Jhoria	G ₈
37.		Mahrajganj	G ₉
38.		Arakhpur	G ₁₀
39.	Varanasi	BHU farm-1	V ₁
40.		Cholapur	V ₂
41.		Danganj	V ₃
42.		Gopalpur	V ₄
43.		Jansa	V ₅
44.		Chaubeypur	V ₆
45.		Pindra	V ₇
46.		Daphi	V ₈
47.		Jagatganj	V ₉
48.		BHU Farm-2	V ₁₀

Table.2 Cultural and Morphological characters of different isolates of *R. solani*

Isolates	Growth rate	Hyphal diameter (µm)	Colony colour	Zonation
A ₁	1.14	9.09	Light grey	Present
A ₂	1.25	7.27	Light pale brown	Present
A ₃	1.25	7.77	Dark pale brown	Absent
A ₄	1.16	7.48	Light yellowish brown	Absent
A ₅	1.06	8.99	Light grey	Absent
A ₆	0.83	6.32	Light yellowish brown	Absent
A ₇	1.18	8.24	Dark pale brown	Present
A ₈	1.16	9.25	Light pale brown	Absent
A ₉	1.25	7.08	Light Pale brown	Present
A ₁₀	1.15	7.23	Dark pale brown	Absent
B ₁	0.99	5.13	Pinkish white	Absent
B ₂	1.25	6.08	Dark Pale Brown	Absent
B ₃	1.14	7.05	Dark pale brown	Present
B ₄	1.23	6.05	Yellowish white	Absent
B ₅	1.14	7.08	Light yellowish brown	Absent
B ₆	1.04	7.18	Light pale brown	Present
B ₇	0.91	8.05	Dark pale brown	Absent
B ₈	0.73	7.05	Light grey	Absent
G ₁	1.20	7.50	Light pale brown	Absent
G ₂	1.25	7.55	Yellowish white	Absent
G ₃	1.14	6.95	Light pale brown	Present
G ₄	0.85	7.23	Light grey	Absent
G ₅	0.80	7.70	Light yellowish brown	Absent
G ₆	1.03	10.21	Light grey	Absent
G ₇	1.19	7.75	Light pale brown	Absent
G ₈	0.80	8.49	Dark pale brown	Absent
G ₉	1.25	6.80	Light pale brown	Present
G ₁₀	1.00	6.93	Light pale brown	Absent
J ₁	1.10	7.08	Light pale brown	Present
J ₂	0.95	7.55	Light grey	Absent
J ₃	1.25	6.29	Dark pale brown	Absent
J ₄	1.17	6.41	Dark pale brown	Present
J ₅	0.98	6.30	Light pale brown	Present
J ₆	0.98	7.14	Dark pale brown	Absent
J ₇	0.98	7.46	Dark pale brown	Absent
J ₈	1.16	7.86	Yellowish white	Absent
J ₉	1.25	8.47	Yellowish white	Absent
J ₁₀	1.17	6.95	Light grey	Present
V ₁	1.19	7.77	Light pale brown	Present
V ₂	1.24	5.72	Yellowish white	Absent
V ₃	0.90	7.50	Dark pale brown	Absent
V ₄	1.24	7.41	Yellowish white	Absent
V ₅	1.25	5.53	Dark pale brown	Present
V ₆	1.14	6.60	Dark pale brown	Present
V ₇	1.25	7.78	Yellowish White	Present
V ₈	1.23	7.55	Light yellowish brown	Absent
V ₉	1.25	7.46	Dark pale brown	Present
V ₁₀	1.05	7.23	Light Pale brown	Present

Table.3 Sclerotial variability of different isolates of *R. solani*

Isolates	Initiation of sclerotia formation (hrs)	Colour of sclerotia	Pattern of sclerotia formation	Location of sclerotia formation
A ₁	84	Radish brown	On periphery	Surface
A ₂	72	Medium brown	Throughout plate	Surface
A ₃	72	Dark brown	On periphery	Surface
A ₄	84	Dark brown	Throughout plate	Surface
A ₅	84	Medium brown	Throughout plate	surface/Aerial
A ₆	96	Medium brown	On periphery	Surface
A ₇	84	Light brown	Concentric rings	Surface
A ₈	84	Light brown	Rings near inoculation point	Aerial/ Surface
A ₉	72	Light radish brown	Throughout plate	surface/Aerial
A ₁₀	84	Dark brown	Throughout plate	Aerial
B ₁	96	Reddish brown	Concentric rings	surface/Aerial
B ₂	72	Dark Brown	Throughout plate	Aerial
B ₃	84	Dark brown	Throughout plate	Surface
B ₄	84	Medium brown	Concentric rings	Surface
B ₅	84	Dark brown	On periphery	Surface
B ₆	84	Dark brown	On periphery	Surface
B ₇	96	Reddish brown	Throughout plate	Surface
B ₈	102	Light brown	On periphery	Surface
G ₁	84	Dark brown	Throughout plate	surface/Aerial
G ₂	72	Light brown	Throughout plate	Aerial
G ₃	84	Light brown	On periphery	Surface
G ₄	96	Light brown	Throughout plate	Surface
G ₅	102	Dark brown	Concentric rings	surface/Aerial
G ₆	84	Light brown	Throughout plate	Surface
G ₇	84	Reddish brown	On periphery	Surface
G ₈	102	Dark brown	Throughout plate	Surface
G ₉	72	Light brown	Throughout plate	Aerial
G ₁₀	84	Dark brown	On periphery	Surface
J ₁	84	Dark brown	On periphery	Partially embedded
J ₂	96	Dark brown	Throughout plate	Partially embedded
J ₃	72	Light brown	Throughout plate	Surface
J ₄	84	Dark brown	On periphery	Surface
J ₅	96	Dark brown	Rings near inoculation point	Surface
J ₆	96	Medium brown	On periphery	Surface
J ₇	96	Dark brown	Throughout plate	surface/Aerial
J ₈	84	Light brown	Throughout plate	Surface
J ₉	72	Radish brown	On periphery	Surface
J ₁₀	84	Medium brown	On periphery	Partially embedded
V ₁	84	Dark brown	On periphery	Surface
V ₂	84	Dark brown	Rings near inoculation point	Surface
V ₃	96	Reddish brown	Concentric rings	surface/Aerial
V ₄	84	Reddish brown	On periphery	Surface
V ₅	72	Dark brown	On periphery	Surface
V ₆	84	Radish brown	Throughout plate	Surface
V ₇	72	Radish brown	On periphery	Surface
V ₈	84	Dark brown	Concentric rings	Surface
V ₉	72	Dark brown	Throughout plate	Surface
V ₁₀	84	Radish brown	Throughout plate	Aerial

Table.4 Sclerotial variability and virulence of different isolates of *R. solani*

Isolates	Number of sclerotia/plate*	Size of sclerotia (mm)	Virulence (%)	Category
A ₁	75.00	0.84	84.09	HV
A ₂	108.66	0.93	88.61	HV
A ₃	164.00	1.65	79.27	MV
A ₄	243.00	2.65	87.89	HV
A ₅	96.66	1.75	66.87	MV
A ₆	116.66	1.80	50.15	MV
A ₇	210.00	0.71	85.69	HV
A ₈	56.67	1.43	71.76	MV
A ₉	74.00	1.83	84.38	HV
A ₁₀	57.33	1.12	84.60	HV
B ₁	63.00	0.56	58.51	MV
B ₂	81.33	2.35	88.35	HV
B ₃	123.00	0.78	85.53	HV
B ₄	71.00	1.87	85.21	HV
B ₅	164.00	0.83	79.71	MV
B ₆	73.00	1.25	60.23	MV
B ₇	117.00	1.53	84.81	HV
B ₈	110.00	0.95	75.65	MV
G ₁	72.33	1.32	67.39	MV
G ₂	110.00	0.94	73.21	MV
G ₃	63.33	1.42	46.24	LV
G ₄	106.00	0.79	80.21	HV
G ₅	111.00	1.80	79.71	MV
G ₆	100.00	0.98	63.46	MV
G ₇	147.00	1.65	54.32	MV
G ₈	117.33	1.28	65.03	MV
G ₉	67.33	2.20	35.57	LV
G ₁₀	216.60	1.25	55.54	MV
J ₁	45.66	0.82	78.38	MV
J ₂	89.00	1.27	65.97	MV
J ₃	70.00	1.50	78.46	MV
J ₄	62.00	1.35	71.88	MV
J ₅	122.00	1.33	92.15	HV
J ₆	113.50	0.75	54.32	MV
J ₇	119.68	0.78	77.94	MV
J ₈	82.00	1.50	54.03	MV
J ₉	76.00	0.94	81.77	HV
J ₁₀	62.00	2.26	88.70	HV
V ₁	107.66	0.89	83.00	HV
V ₂	75.33	1.45	81.56	HV
V ₃	158.00	1.52	55.40	MV
V ₄	150.00	2.20	83.03	HV
V ₅	96.66	0.95	59.74	MV
V ₆	210.00	0.93	87.84	HV
V ₇	133.50	0.76	77.35	MV
V ₈	40.66	0.77	55.57	MV
V ₉	132.50	0.65	89.05	HV
V ₁₀	53.33	0.55	33.18	LV

*Average of four replications

The detailed data of sclerotial variability for 48 isolates has been given in table 3 and 4. The isolates depicted wide range of virulence per centage and it ranged from 33.18 % (V₁₀) to 92.15 % (J₅). Based on the virulence, 19 isolates were categorized as highly virulent, 26 isolates were considered moderately virulent and only three isolates were found less virulent. The detailed data for size of sclerotia and virulence of different isolates have been given in table 4.

In the past Basu *et al.*, (2004); Lal and Kandhari (2009) and Mishra *et al.*, (2014) have also reported variation in growth rate of different isolates of *R. solani*. They have also reported various colony colour of *R. solani* which ranged from brown, light brown, dark brown, yellowish brown and whitish brown. The discolouration of the growth media is mainly attributed to the production of pigments by the pathogens. The differences in the intensity of the colour may also correspond to the amount of pigments released by respective isolates in the media. The absence and presence of zonation identified in the present investigation in the cultures of *R. solani* has also been reported by Singh *et al.*, (1999). Isolates of *R. solani* are known to show greater variability in virulence (Windles and Nabben, 1989). Differences in virulence of *R. solani* isolates collected from various hosts and geographical areas have been reported by several other workers (Phillips, 1991 and Lehtonen, 2009).

Enormous degree of variation in virulence of 48 isolates of pathogen was demonstrated in the present study. Similarly Tiwari and Khare (1998) had also grouped *Rhizoctonia solani* isolates of mungbean into three different categories of poorly virulent, moderately virulent and highly virulent on the basis of the degree of their virulence. The present investigation revealed that isolates of *R. solani* greatly varied in morphological,

cultural, sclerotial characteristics and virulence. It can be argued that variation in the isolates may be inherent since isolates were collected from different locality; hence the morphological and physiological characters are influenced by environmental conditions through natural chance mutations which may be responsible for such variability.

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