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CULTURAL AND MORPHOLOGICAL CHARACTERIZATION OF THE PATHOGEN (*Rhizoctonia solani* L.) CAUSING WEB BLIGHT OF MUNGBEAN

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ABSTARCT

A study was carried out to characterize isolates of pathogen causing web blight in mungbean by adopting the morphological and cultural features. The isolates were recovered from diseased plant samples representing different locations of Punjab. In microscopic study, all the isolates exhibited typical characters of *Rhizoctonia* e.g. right-angle branching near the distal septum and constriction at the base of branching. Cultural and morphological study revealed that *Rhizoctonia* isolates differed in terms of mycelial colour, texture, growth rate, number and colour of sclerotia. The mycelial colour of five isolates varied from cream brown to dark brown. Ballowal Saunkhri (BS) and Nawanshahr (NS) isolates showed fast growth (0.88 mm/h) while Ludhiana isolates (L1, L2) were slow growing (0.81, 0.68 mm/h). Mycelial texture was flat in all isolates except Ropar (RO) isolate which was semi cottony. Mycelial width ranged from 4.74 µm (L1) to 6.43 µm (NS). Along with isolate BS, L2 produced the highest number of sclerotia (>60/plate) in spite of its slowest growth rate. Vegetative compatibility was observed in isolates combination of L1-L2, BS-L1, RO-L1, NS-L1 and NS-BS. The outcomes of present study related to growth rate and ability of sclerotia production may be used in future research to correlate with the pathogen's virulence.

Keywords: Anastomosis, Leaf blight, Rhizoctonia, Variability, *Vigna radiata*

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Mungbean is considered a major pulse crop after chickpea and pigeon pea in India. It constitutes more than 70 percent of total mungbean production of the world (Agricultural Market Intelligence Centre PJTSAU, 2021). In Punjab, area under mungbean was estimated around 3.3 thousand hectares during 2023-24 with production and productivity of 3.3 thousand tonnes and 9.96 q/ha, respectively (Anonymous, 2025). The production of mungbean is arrested by various fungal, bacterial and viral diseases. Among fungal diseases, Web blight (*Rhizoctonia solani*) is one of the major diseases, which appears every year at varying intensity causing huge losses (Singh *et al.,* 2017; Biswas and Banerjee, 2019) in India. It was initially reported in India by Dwivedi and Saksena (1974) from Kanpur, Uttar Pradesh. *Rhizoctonia solani* subsists mainly as vegetative mycelium and/or sclerotia in natural environment. Like other fungal genera, *Rhizoctonia* was initially recognized based on morphological and cultural characters. The hyphal branching at right angle, constriction at the base of branching and more than three nuclei per cell of hypha reveal the characteristic features of the genus *Rhizoctonia* (Hawksworth *et al.*, 1995). Furthermore, the *R. solani* is presently placed in 14 anastomosis groups on the basis of hyphal fusion (anastomose), cultural and morphological characters, pathogenicity or virulence and DNA homology (Godoy-Lutz *et al.,* 2003; Harikrishnan and Yang, 2004).

Although breeding for disease resistance is a realistic and viable strategy for disease management, however it may not serve as a definitive solution in case of *R. solani*. Because the potential diversity in cultural and morphological traits was exhibited among *R. solani* isolates (Lal and Kandhari, 2009; Singh *et al*., 2018) that may have an impact on disease management. Even though numerous studies have been made pertaining to morphological variations of *R. solani* (Mishra *et al*., 2014; Singh *et al*., 2014; Madhavi *et al*., 2015; Rajput and Harlapur 2016; Gopireddy *et al*., 2017; Singh *et al*., 2018), there is still a paucity of information about the cultural and morphological variations in *R. solani*, particularly infecting the mungbean crop and causing web blight. Moreover, several studies on growth rate, reproduction and sclerotia production in fungal pathogens are positively correlated with virulence whereas other studies indicate contrary relationship (Chen *et al.*, 2004; Meyer *et al*., 2010; Uddin *et al.*, 2023). Keeping in view the above facts and economic importance of web blight pathogen of mungbean, it has to be characterized correctly. Therefore, purpose of the current study was to investigate morphological and cultural diversity among *R. solani* isolates of mungbean

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that were recovered from diseased samples from various locations of Punjab.

MATERIALS AND METHODS

Isolation

The diseased leaves of mungbean plants exhibiting typical symptoms of web blight and sclerotia were also collected from Pulses Research Farm of PAU, Ludhiana, Dr. D.R. Bhumbla Regional Research Station at Ballowal Saunkhri, Nawanshahr and Ropar (Table 1) for isolating the fungus. The diseased leaf samples were cleaned with water and cut into 5-6 mm pieces with a sterile blade. Then leaf pieces were surface sterilized with 2 percent sodium hypochlorite solution for 1 min and washed thrice with sterile distilled water to remove excess of chemical. After surface drying, pieces of diseased tissues were aseptically transferred on PDA and incubated at 27 ± 2°C for 24h. Later, pure cultures for all isolates were obtained by hyphal tip culture method (Rangaswami and Mahadevan, 2004). To obtain a pure culture, a single hyphal tip was cut from the edge of a fungal colony on a Petri plate using a sterile needle. The hyphal tip was isolated from the farthest point of the main colony, confirming it as a single hyphal filament. Then tip was placed aseptically in the center of the agar plate to allow for even growth. The plate was incubated at 27±2°C temperature in a BOD incubator for 3-5 days to get a pure culture from single hyphal tip. Sub culturing was also done on PDA slants at regular intervals to maintain the purity of culture. The pure cultures were stored in refrigerator at 4° C for further studies. After establishing the pathogenicity of the isolated fungus, the cultural and morphological characteristics were studied in details.

Microscopic examination

All the five different isolates (Table 1) of web blight pathogen collected from three districts (SBS Nagar, Ludhiana and Ropar) were examined under microscope to see the peculiar identification features of the pathogen. Microscopic inspection was performed using a temporary slide prepared in lacto-phenol. The isolates were recognized by morphological characters and already published literature (Parmeter and Whitney 1970; Sneh *et al*., 1991) using the characteristic keys of *Rhizoctonia solani viz*. i) young vegetative hyphae possessing multinucleate cells and branch towards the distal septum ii) a noticeable septal spore apparatus iii) formation of a septum in a branch near its origin iv) constriction at the base of branch and v) shade of brown pigmentation.

Cultural and morphological characters

The isolated fungus was sub-cultured on PDA Petri plates incubated at 27±2°C. The mycelial bit (7mm) of each isolate from a seven-day old culture was placed aseptically in triplicates at the center of a Petri plate (90mm) containing PDA. The observations related to various morphological and cultural characteristics were recorded for seven days of inoculations. The colony growth at the interval of 24h, 48h, 72h, 96h, 120h, 144h and 168h (Kumar *et al*., 2019), colour of the culture (Moni *et al*., 2016) and mycelial abundance (Susheela, 2012; Lal *et al*., 2014) were recorded. The observations were also noted down for pattern (Singh *et al*., 2014), colour (Moni *et al*., 2016), number (Lal *et al*., 2014; Kipsumbai *et al*., 2022) and size of sclerotia using software Mi CAM 2.0. photographs. Different categories of sclerotia were explained as per details given by Goswami *et al.* (2019).

RESULTS AND DISCUSSION

Microscopic examination and identification

All the isolates obtained from diseased leaf samples of mungbean, namely L1 (Ludhiana 1), L2 (Ludhiana 2), BS (Ballowal Saunkhri), NS (Nawanshahr) and RO (Ropar) were examined under a microscope. In microscopic study under 40X of magnification using Mi Cam 2.0 on computer, the young hyphae appeared septate and hyaline however, it turned brown with growth and maturity. Mycelium had a diameter ranging from 4-9 µm. However, the diameter of hyphae changed from young to old. Young hyphae had tapered branches formed at 45° angles but mature branches were perpendicular and the same size. There was slightly narrowed point (constriction) at the base of branch as well as a septum (Fig. 1). The above-mentioned features are typical characters of *R.*

Table 1. List of *Rhizoctonia* isolates collected from web blight infected mungbean fields in selected districts

Sr. No.	Location	District	Variety	Isolate name
1	PAU Pulses Farm	Ludhiana	ML-2056	L
2	PAU Plant Pathology Farm	Ludhiana	ML-2056	L2
3	RRS, Ballowal Saunkhri	SBS Nagar	ML-2056	BS
4	Nawanshahr	SBS Nagar	ML-2056	NS
5	Ropar	Ropar	ML-2056	RO

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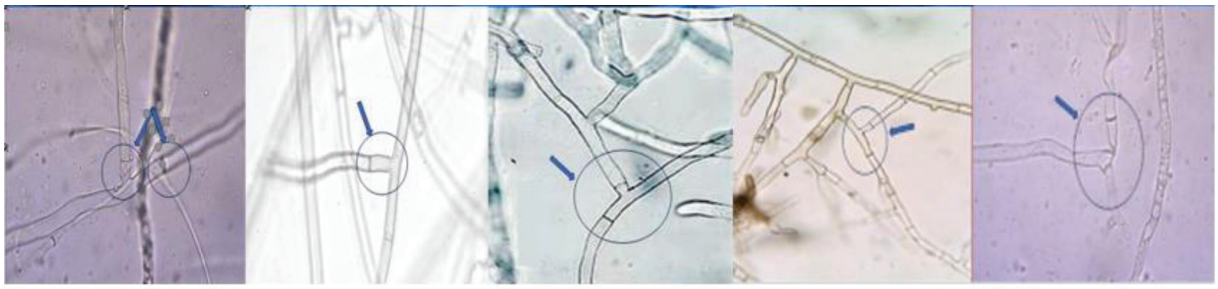


Fig. 1. Microscopic examination of *Rhizoctonia solani* isolates (L1, L2, RO, NS, BS)

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solani which are crucial for identification of the genus. Based on above mentioned three traits exhibited by all of the isolates during study and already published literature (Parmeter and Whitney, 1970; Talbot, 1970), these cultures were identified as *Rhizoctonia solani*. In addition to above typical features, other workers had also reported multinucleated cells, a dolipore septum in growing hyphae and oval to ellipsoidal monilioid cells in mycelium prior to the production of sclerotia in case of *R. solani* isolates (Sahu 2003; Abdel-Sattar *et al.,* 2017; Sandoval and Cumagun, 2019).

Cultural and morphological characterization

a. Mycelial characteristics of Rhizoctonia *isolates*

Mycelial colour: The mycelial color of five isolates appeared from cream brown/faint brown (CB) to dark brown (DB) based on the color intensity. Among five isolates, the mycelial color appeared to be same for both Nawanshahr (NS) and Ropar (RO) isolates, whereas rest of the three isolates i.e., BS, L1, L2 showed cream brown, medium brown and dark brown color, respectively (Table 2). Nearly similar type of observations related to colony color as brown, light brown, dark brown and yellowish brown of *Rhizoctonia* isolates were noticed by several workers (Lal and Kandhari, 2009; Debbarma and Dutta, 2015; Singh and Kumar, 2018).

Mycelial abundance: Based on the mycelial abundance (Table 2), isolates were categorized as Abundant (A), Moderate (M) and Slight (S). Our results showed that all isolates except NS exhibited abundant mycelium (Table 2). However, none of the isolates under study showed slight mycelial abundance. Similar growth patterns of mycelium have been observed in isolates obtained from mungbean (Susheela, 2012; Lal *et al*., 2014) and rice (Pralhad *et al*., 2019) crop in earlier studies. In further studies, an extensive, sparse and moderate growth of mycelium was also documented (Rajput and Harlapur, 2016; Sandoval and Cumagun, 2019).

Table 2. Mycelial characteristics of *Rhizoctonia* isolates

	Mycelial characteristic					
Isolate	**Mycelial color (cream/ light/ medium/ dark brown) (CB/LB/MB DB)**	**Mycelial abundance: abundant (A), moderate (M), slight (S)**	**Quality of aerial mycelium: absent (0), all hyphae close to surface of agar (0.1-1), plate half filled (1.1-2), almost all filled (2.1-3)**	**Mycelial texture: flat (F), semi cottony (SC), cottony (C)**	**Mycelial dispersion: aggregate (A), moderate (M), spatial (S)**	**Mycelial width (µm)**
BS	CB	A	0.66	F	M	5.20[a]
L1	MB	A	1	F	A	4.74[a]
L2	DB	A	1.33	F	M	4.82[a]
RO	LB	A	1	SC	S	6.10[b]
NS	LB	M	1	F	S	6.43[b]
CD (*p*=0.05)						0.47
CV (%)						5.70

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Aerial mycelial quality: The observation on quality of aerial mycelium was based on the coverage of Petri plates by the colony. Isolates L1, BS, RO and NS hyphae were close to the surface, whereas isolate L2 mycelium were present within half portion of the Petri plate with value 1.33 (Table 2). In earlier studies on *R. solani* isolates recovered from urdbean, mungbean, maize and rose, aerial mycelial quality in the colonies was found moderate (Moni *et al.,* 2016).

Mycelial texture and dispersion: The mycelial texture was categorized as flat (F), semi-cottony (SC) and cottony (C). All isolates except RO (Ropar) isolate showed flat texture of mycelia, whereas RO exhibited semi cottony growth. The L1 isolate formed aggregate mycelium, while BS and L2 isolates had moderate dispersion. The RO and NS, on the other hand, showed spatial dispersion of mycelium (Table 2). These results are in accordance with Mishra *et al.* (2015) and Goswami *et al.* (2019)**.**

Mycelial width: Dino Capture 2.0 was used to estimate the hyphal width of 48-hour-old cultures. Almost equal average mycelial width of 4.74 µm and 4.82 µm was recorded in L1 and L2 isolates, respectively. The isolate BS had a width of 5.20 µm, whilst the isolate RO and NS had mycelial widths of 6.10 µm and 6.43 µm, respectively (Table 2). The isolates BS, NS & RO and L1 & L2 were statistically at par in terms of mycelial width (µm). More or less similar range of hyphal width were also noticed by Gopireddy *et al.* (2017) and Sandoval and Cumagun (2019) during characterization of different isolates of *Rhizoctonia* spp.

Colony growth rate: The *Rhizoctonia* isolates were divided into three groups based on their colony growth as slow, medium and fast (Fig. 3). The highest colony growth rate (0.88 mm/h) was recorded for isolates BS and NS indicating the fast growing isolates, whereas L2 isolate was slow growing with least growth rate (0.68 mm/h). Isolates RO and L1 exhibited intermediate growth rate of 0.82 mm/h and 0.81 mm/h, respectively. In case of colony growth rate, the isolates BS, NS, RO and L1 were statistically at par except isolate L2 which exhibited more than 0.8 mm colony growth per hour. In future studies, the current growth rate findings could be used to correlate with the virulence of the pathogen. The results are in accordance with earlier studies conducted by Susheela and Reddy (2013), Desvani *et al.* (2018) and Sandoval and Cumagun (2019). They also grouped the *R. solani* isolates into fast-growing, moderate-growing and slow-growing isolates. Other studies based on colony growth rate per day classified the *R. solani* isolates as rapid, moderate and slow growing isolates (Guleria *et al.*, 2007; Jayaprakashvel and Mathivanan, 2012; Lata *et al.*, 2018; Nagaraj *et al.*, 2019).

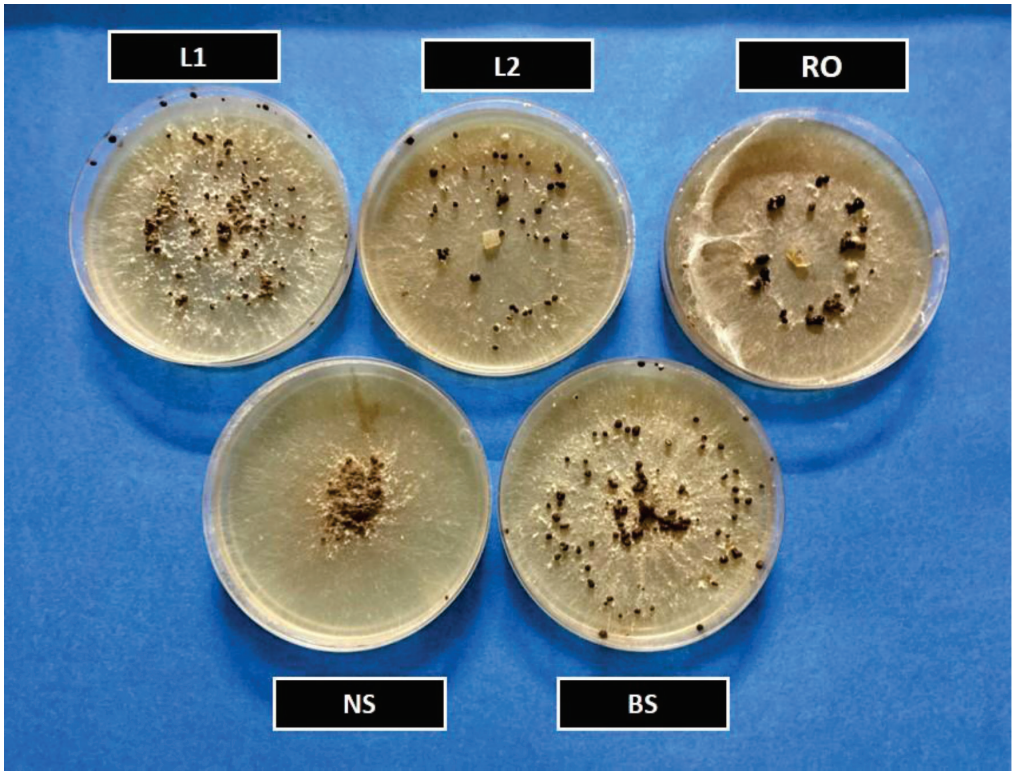


Fig. 2. *Rhizoctonia solani* isolates L1 (Ludhiana 1), L2 (Ludhiana 2), RO (Ropar), NS (Nawanshahr), BS (Ballowal Saunkhri) on Petri plates

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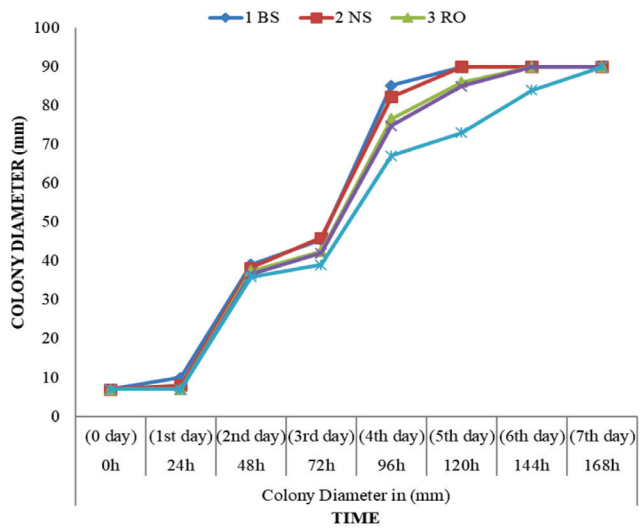


Fig. 3. Colony growth rate of *Rhizoctonia solani* isolates

b. Sclerotial characteristics of Rhizoctonia *isolates*

Sclerotia were developed in all of the isolates (L1, L2, BS, NS and RO) and varied in number and size (Fig. 2). A thin piece of sclerotium has a large number of isodiametric cells with thicker walls and darker outer cells. In culture and host tissue, sclerotia were measured as 1.0-2.0 mm and 1.0-1.5 mm in diameter, respectively. The size of sclerotia from culture was larger than those from spontaneously infected hosts. The sclerotial characteristics so observed are shown in table 3 and 4.

Time taken for initiation of sclerotia formation: Different isolates were monitored in order to determine how long time it takes for sclerotial development. The isolates BS and NS took approximately 6 days, while isolates RO, L1 and L2 each took one day longer i.e. 7, 8 and 9 days, respectively for the development of sclerotia (Table 3). The L1 & L2 isolates were statistically at par where sclerotia development initiated after one week of inoculation while isolates BS, NS and RO took less than a week for initiation of sclerotia development and were at par. The quicker sclerotia producing isolates could be compared with slower sclerotia producing isolates in terms of their virulence in future studies. The results of sclerotial development are in accordance with Goswami *et al*. (2019) who categorized all isolates of *R. solani* into three groups i.e. fast (4 days), moderately fast (4-5 days) and slow (>5 days) based on time required to develop the sclerotia.

Sclerotial colour: Based on observations, sclerotial colour was categorized into four: cream brown (CB), light brown (LB), medium brown (MB), and dark brown (DB). On an average, the sclerotia of isolates BS, L1, L2, and RO had dark brown (DB) color, whereas NS isolate was with medium brown sclerotia (Fig. 2 & Table 3). More or less similar colour of sclerotia was also noticed in case of rice sheath blight pathogen (Moni *et al.*, 2016; Singh *et al.*, 2018; Pralhad *et al.*, 2019).

Number of sclerotia per plate: All the five isolates produced different number of sclerotia per Petri plate (Table 3). The isolates BS & L2 were statistically at par and excellent sclerotia producers (>60 sclerotia/plate) while isolates RO & NS (25.67 and 30.00, respectively) were statistically at par and good in sclerotia production. The aforesaid findings are supported by Upadhyay *et al.* (2013) who classified *R. solani* isolates

Table 3. Sclerotial characteristics of *Rhizoctonia solani*

Sclerotial characteristic						
Name/ code of isolate	**Duration for initiation of sclerotia development (Days)**	**Number of sclerotia per plate: 0 (poor), 1-10 (fair), 11-20 (mod), 21-40 (good), 41-60 (very good), >60 (excellent)**	**Sclerotia on underside of lid: P-Present, AB-absent**	**Sclerotial colour (CB-Cream brown faint brown/ LB-light brown/ MB-medium brown/ DB-dark brown)**	**Topography: I-immersed, S-superficial**	**Location of sclerotia: A-aerial s-surface e-embedded)**
BS	6[a]	62.33[a]	P	DB	S	S+A
L1	8[b]	36.67[b]	P	DB	S	A+S
L2	9[b]	61.67[a]	P	DB	S	S
RO	7[a]	25.67[c]	AB	DB	I+S	S+E
NS	6[a]	30.00[c]	AB	MB	S+I	E+S
CD (*p*=0.05)	1.0	5.45				
CV (%)	8.8	8.28				

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Table 4. Sclerotial characteristics of *Rhizoctonia solani*

Name/code of isolate	Sclerotial characteristic			
	Pattern of sclerotia (1=On periphery, 2=throughout plate, 3=ring near inoculation point, 4=concentric rings)	Sclerotial shape (0=flattened bottom round top, 1=superficial, 2=irregular globose pitted surface, 3=irregular)	Size of sclerotia (>1.25 mm=macro, ≤ 1.25 mm=micro)	Sclerotial clump formation more (>16), moderate (11-16), less (1-10), absent (Ab)
BS	2	1+0+2	1.040	13 (Mod)
L1	2	0+1+3	1.063	4 (Less)
L2	2	1+3	1.068	12.67 (Mod)
RO	3	0+1+3	2.174	18.33 (More)
NS	at tip	2+3+1+0	-	2 (Less)

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as greater (>400 sclerotia), medium (>200 to 400 sclerotia) or lower (200 sclerotia) based on the number of sclerotia produced per plate. In another study, *Rhizoctonia* isolates were categorized into four groups (>9.0=more, 5.1-9.0=moderate, ≤5.0=less, and absent) where sclerotial number counted under a binocular microscope 7 days after inoculation (Goswami *et al.*, 2019). An interesting observation was also recorded that isolates BS & L2 both were excellent sclerotial producers, however L2 exhibited slow growth rate and took longer duration for the development of sclerotia as compared to BS. The strong correlation was observed between sclerotia production and virulence of fungal plant pathogens in earlier studies. Chen *et al.* (2004) reported that mutants of *Sclerotinia sclerotiorum* which were deficient in sclerotia production, exhibit decreased virulence. Therefore, present finding had given an idea for future research to correlate the sclerotia producing efficiency of the pathogen with its virulence.

Sclerotia present on underside of lid: Sclerotial production can also be observed on the underside of the lid under aseptic conditions in the laboratory (Table 3). Sclerotia of isolates BS, L1 and L2 were found on the undersides of the lids denoted with letter 'P', whereas isolates RO and NS did not develop sclerotia on the undersides of the lids 'AB'.

Topography and location of sclerotia: The isolate BS produced sclerotia in both surface and embedding situations, while L1 formed at surface and aerial section. Sclerotial development in isolate L2 was on the surface; however, isolates RO and NS were in embedded and surface situations (Table 3). The current study related to texture and distribution pattern of sclerotia of *R. solani* is also supported with the findings of Rashmi *et al.* (2024).

Shape of sclerotia: In Petri plate, the sclerotia were varied in shape for all isolates which were classified in 4 types depicted by numerical values (Table 4). However, Nawanshahr (N) isolate was the only one which had all four shapes of sclerotia. The findings of Aghunandana and Prashanthi (2023) also corroborate the current study related to texture and distribution pattern of sclerotia of *R. solani*.

Size of sclerotia: Based on size, sclerotia were classified as macrosclerotia (>1.25 mm) and microsclerotia (≤ 1.25 mm). The sclerotial size of isolates BS, L1, L2 and RO varied from 1.040-2.174 mm fell under both the categories of microsclerotia as well as macrosclerotia (Fig. 4 & Table 4). However, the sclerotial size of isolate NS was difficult to measure as of typical clump formation. Earlier study conducted by Pralhad *et al.* (2019) is in line with our findings who stated that diameter of sclerotia in web blight isolates ranged from 0.2-5.0 mm.

Distribution pattern of sclerotia: Sclerotia of isolates BS, L1 and L2 showed similar pattern of sclerotial distribution i.e. throughout the plate (2) while sclerotia of isolate RO formed ring (3) near the inoculation point (Table 4). The aforesaid results of sclerotial arrangement are in accordance with Susheela and Reddy (2013), Goswami *et al.* (2019), Sandoval and Cumagun (2019) who observed the central, peripheral, dispersed and a ring pattern of sclerotia distribution.

Sclerotial clump formation: The number of sclerotial clumps was lower in isolates NS and L1 with 2 and 4, respectively, whereas isolate RO made a higher number (18.33) of sclerotial clumps (Table 4). These results are in agreement with findings of Goswami *et al.* (2019).

c. Vegetative compatibility

The vegetative compatibility of the five isolates was tested in all feasible combinations. On the basis of mycelial compatibility, the five isolate combinations i.e. L1 & L2 (Ludhiana 1 and Ludhiana 2), BS & L1 (Ballowal Saunkhri and Ludhiana 1), RO & L1 (Ropar and Ludhiana 1), NS & L1 (Nawanshahr and Ludhiana 1) and NS & BS (Nawanshahr and Ballowal Saunkhri) were vegetatively compatible. However, rest of the

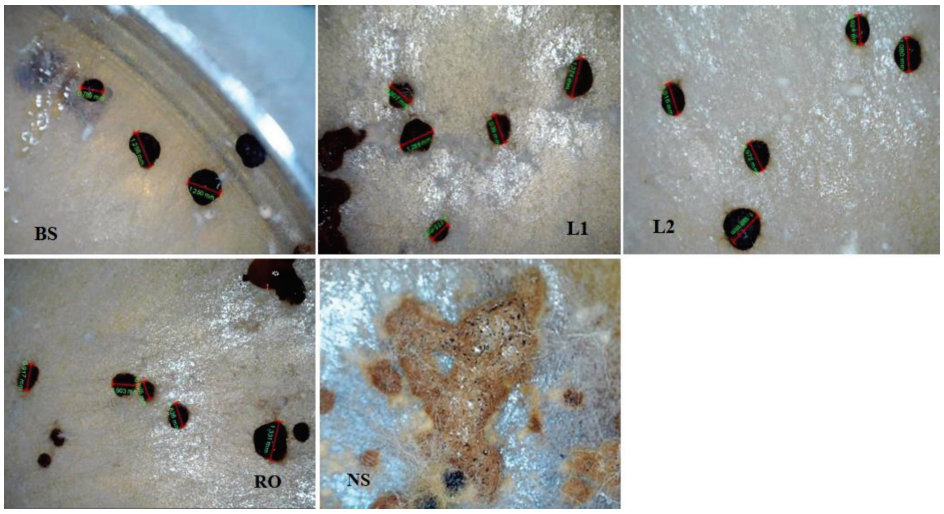


Fig. 4. Measurement of sclerotial size of *Rhizoctonia solani* isolates

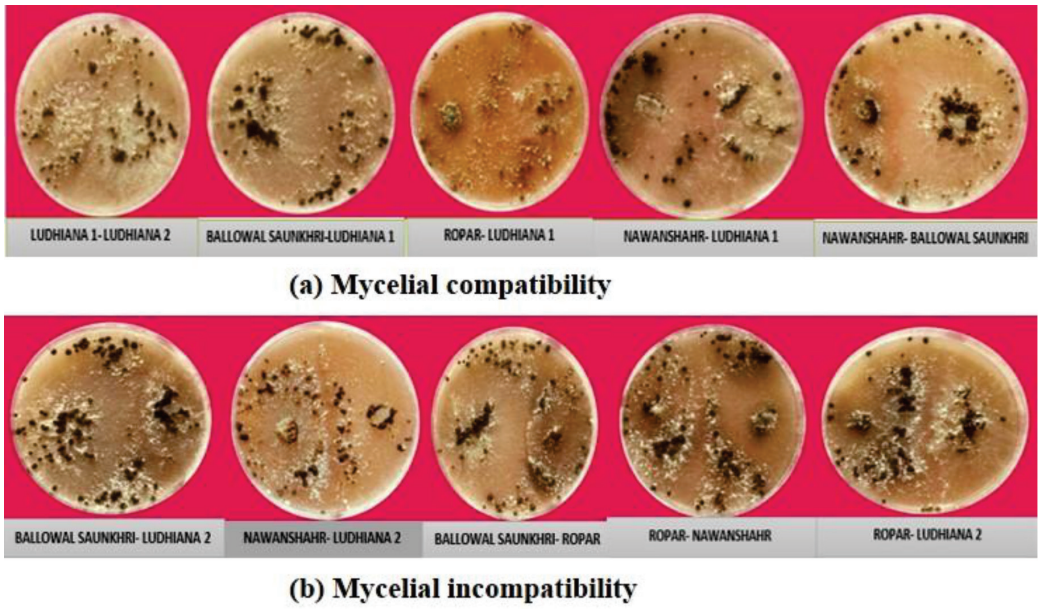


Fig. 5. Hyphal compatibility (a) and incompatibility (b) of *Rhizoctonia solani* isolates

combinations revealed mycelial incompatibility due to visible demarcations between paired isolates (Fig. 5). In several studies, mycelial compatibility of *R. solani* isolates belonged to 21 AG-1-IE and 13 AG-1-IF group (Gonzalez *et al*., 2012), AG-3 group isolates of potato and tobacco (Ceresini *et al.,* 2002) and AG-1-IA isolates of soybean (Campos and Ceresini, 2006) was observed.

CONCLUSION

Cultural and morphological characteristics are important features for identifying the fungi and assessing their variability. Present study revealed the significant variation both in macroscopic and microscopic characteristics of *R. solani* isolates. The isolates were identified based on their morphological characters during the study. Ballowal Saunkhri isolate (BS) was fast growing (0.88 mm/h) and produced maximum number of sclerotia (>60/plate). This isolate was also reported to cause severe disease in the field. The findings of present study on growth rate and ability of sclerotia production may be correlated with virulence of the pathogen which could help to learn more about the nature of pathogen in future research.

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Authors' contribution

Conceptualization of research work and design of experiment (KKS, AS & RS), Execution of field/ lab experiments and data collection (RS, AS & KKS), Analysis of data and interpretation (RS, KKS & AS), Preparation of Manuscripts (KKS, AS & RS).

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