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Morphological and pathogenic variability of root rot of soybean in western Maharashtra caused by *Rhizoctonia bataticola*

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Abstract
Root rot caused by *Rhizoctonia bataticola* (Taub.) Butler (Synonym: *Macrophomina phaseolina* (Tassi) Goid) is a fungus that is observed all over the world and is a serious pathogen in many crops. In the present study, Twenty isolates of root rot of soybean were collected from soybean growing tahsils of ten districts of Western Maharashtra during the *kharif* season of 2017-18. It was found that the root rot disease of soybean was predominant in all districts but their incidence was varied. The survey found that disease incidence was higher in rainfed crops than in irrigated crops. The dry conditions prevalent in rainfed areas may have favored the pathogen, which could be attributed to the higher level of disease incidence. Twenty isolates of *R. bataticola* were characterized for morphological characteristics. The isolates showed a varied pattern of hyphal width, sclerotial characters like shape and size, the average size of sclerotia ranged between 110.24 × 84.11 - 40.71 × 36.21 µm. Pathogenicity of the test pathogen isolates was proved successfully on soybean Cv. JS 335, by sick soil method in pot culture, under screen house conditions, based on which, all of the 20 isolates of *R. bataticola* were grouped into various categories which showed isolates as weakly to highly pathogenic to soybean.

Keywords: *Rhizoctonia bataticola*, soybean, mycelial growth

Introduction
Soybean has a prominent place as the world's most important seed legume, which contributes 25 percent to the global vegetable oil production, Due to its high productivity, profitability and vital contribution towards maintaining soil fertility soybean is a very important crop. About two-thirds of the world's protein is concentrated for livestock feeding and is a valuable ingredient in formulated feeds for poultry and fish. About 85 percent of the world's soybeans are processed annually into soybean meal and oil. Soybean seed contains approximately proteins (37-41%), oil (18-21%), carbohydrates (30-40%) and ash (4-5%). It has high protein and oil content without cholesterol due to this dual quality it is also called a “Golden bean”. Soybean improves soil health and fertility by fixing nitrogen through biological nitrogen fixation in soil which is carried out by symbiotic nitrogen-fixing bacteria residing in the root nodule of soybeans (Javaid and Mahmood, 2010) [10]. Soybean also can ameliorate the nutritional situation, enhance the productivity of other crops and also protects the environment from allelopathy tendencies of agricultural chemicals (FAO, 1998) [5].
In India soybean occupying area is 11.33 million hectares with a production of 13.79 Mt and productivity of 12.17 t/ha in 2019. Production of soybean In India is dominated by Maharashtra and Madhya Pradesh which contribute 86 percent of the total production. In India, Maharashtra is the second-largest soybean producer state which has an area occupying 4.04 million ha with a production of 4.55 Mt and productivity of 11.25 t/ha in 2019. The soybean crop is susceptible to more than 100 pathogens (Sinclair andShurlleff. 1975) [17], but very few of them are economically important and responsible for yield loss (Niblack *et al*. 2004) [14]. Soybean is affected by fungal, bacterial and viral diseases. There are more than 40 fungal pathogens that have been reported that cause infection in soybean worldwide (Hartman, 1999) [6] out of which about 35 pathogens were reported in India which causes around 12 percent losses of total production in India (Gupta and Chauhan 2005) [8]. Among the fungal diseases, root rot of soybean caused by *Rhizoctonia bataticola* causes a yield loss of soybean of around 2-21% (Wrather and Koenning, 2006) [19]. This disease generally infects plant during the seedling stage and remain present in tissue till favorable conditions for growth is developed once water stress occurs due to extended dry weather (soil moisture below 60%)

also temperature reaches 25°-30° the rapid growth of pathogen will be developed due to optimum condition (Dhingra, 1975 [4]. In our country earlier it was a minor disease but in the recent past plant are more susceptible to *Rhizoctonia bataticola* due to longer and repeated droughts during the development stage of the crop (Lodha and Mawar, 2010) [12]. *R. bataticola* pathogen will survive for 2-3 years in seeds as well as for a longer period (2-15 years) on plant residues and/or in soil, and due to its immense inoculum potential, it is nearly impossible to control this disease with a single practice like plant rotation or chemicals treatment. In the Present study collection of disease samples were done from soybean growing areas of Western Maharashtra. After the collection of diseases sample pathogenicity was proved.

Material and Method

The soybean crop is mainly grown in the *kharif* season a field survey was conducted in major soybean growing areas from Western Maharashtra during the *kharif* season 2017-2018 and collected the root rot disease samples. In the selected soybean crop fields, a 10 m^2 area was randomly selected and in that counted a total number of soybean plants and several plants showing typical root rot symptoms were recorded and percent root rot disease incidence was calculated by using the following formula."

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{The total number of plants observed}} \times 100$$

During the survey, different locations were surveyed and disease samples were collected. After collection, the disease samples were brought to a section laboratory and fairly dry to avoid further deterioration by the growth of bacterial and saprophytic fungi. Collected diseased samples were stored in a refrigerator for further studies.

Isolation

Plants showing typical root rot symptoms were washed under running tap water and blot dry. Infected roots were cut into pieces of 5-6 mm size and surface sterilized by dipping in 0.1% mercuric chloride for 1 min. After thorough washing in sterile distilled water up to three washes, the pieces were then moved by using forceps on to sterilized potato dextrose agar (PDA) medium in Petri dishes and incubated at 27 ± 2 ^{0}C to obtain mycelial growth. The hyphal tips from the margins of the resulting colonies were cut with the help of a sterilized 5 mm cork borer and transferred to a Petri dish containing PDA. Colonies that developed from the bits were identified by microscopic observations by taking morphological characters as means of identifying the pathogen. The pure cultures of isolates grown on PDA slants were stored at 4 ± 1^{0}C for further studies.

Morphological characterization

For morphological characters, the mycelial discs of 5 mm diameter were cut from the edge of a seven days old culture and transferred aseptically to a 90 mm Petri dish containing 20 ml PDA media. These plates were incubated at 27 ± 2^{0}C. Each treatment was replicated thrice.On the seventh day after incubation width of hyphal cells, sclerotia size and shape were recorded.

Pathogenicity

The pure culture of each isolate was multiplied on sand sorghum medium for 15 days in the sterilized flask. The full-grown inoculum of each isolate multiplied on sand sorghum medium was mixed @ 50 g/kg soil. The earthen pots were sterilized with five percent copper solution and filled with sick (inoculated) soil. Fifteen seeds of soybean Cv. JS-335 was sown in each pot. One-pot was used for each isolate. Each isolate was replicated three times. The seed sown in sterilized (un-inoculated) soil served as control. The pots were kept in glasshouse conditions. The observations on symptoms produced on soybean plants were recorded and the percent disease incidence was worked out by the percent disease incidence (PDI) formula.

$$\text{Percent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Result and Discussion

Collection of Disease samples

For the collection of isolates of root rot of soybean,a survey was undertaken from different tahsils of ten major soybean growing districts of Western Maharashtra *viz*, Satara, Sangli, Kolhapur Ahmednagar, Nashik, Pune, Solapur, Dhule, Nandurbar, Jalgaon, during the *Kharif* season of 2017-18 is presented in (Table 1)

Morphological variability in *Rhizoctonia bataticola*

Twenty isolates of *R. bataticola viz.*, Rb1 to Rb20 collected from different locations of Western Maharashtra were taken for the present study. Variability studies among these isolates of *R. bataticola* were done based on morphology, hyphal width, sclerotial size, the shape of sclerotia. Variations in morphological characteristics were observed in all the isolates of *R. bataticola*.

Hyphal width

Based on the hyphal width of isolates, the pathogen was categorized into three groups. The first group of large hyphal width (>5µm) consisting of 11 isolates, the second group of isolates has medium hyphal width ranging (4-4.99 µm) and the third group with small hyphal width (<4 µm) consists of five isolates. The results were confirmed by the findings of (Jharia and Khare 1985)[11] who studied the variation in hyphal width and reported maximum hyphae width (4.5 µm) in the root isolate, similar effective results were reported by Gupta *et al*. 2012[9] who observed variation in size of hyphal width of 40 *Rhizoctonia bataticola* isolates from chickpea.

Sclerotia size

Based on microscopic observations the sclerotia size varied from 40.71 x 36.21 to 110.24 x 84.11 µm (Table 2). These isolates were categorized into three groups. the first group consists of large sclerotia size (>80 µm) Second group consists of medium sclerotial size ranging from (60 – 80µm), and the third group consists of small sclerotial size (<60 µm). The highest and smallest size was found with Rb11 (110.24 x 84.11 µm) and Rb8 (40.71×36.21 µm) respectively. Similar results were shown by several workers *viz.*, Dhingra, Sharma *et al.* (2006) [15] who studied seven isolates of *M. phaseolina* on maize in which Hyderabad isolate produced the highest number of sclerotia of larger size (95.7 µm) compared to other isolates.

Sclerotial Shape

Based on microscopic observations of the sclerotial shape of the isolates were classified into two groups that are round and oblong. Round shape sclerotia was recorded in isolates Rb 2, Rb 4, Rb 5, Rb 8, Rb 10, Rb 12, Rb 13, Rb 14, Rb 16, Rb 17, Rb 18 and Rb19 whereas, Oblong shape was found in Rb 1, Rb 3, Rb 6, Rb 7, Rb 9, Rb 11, Rb 15, and Rb 20 sclerotia (Table 2). These results are in concurrence with the findings of several researchers Mamta Sharma (2012)[16] who classified the sclerotia of *R. bataticola* into different shapes such as oblong, ellipsoid, irregular and round whereas, Gade *et al.* (2018)[7] classified sclerotia into oblong and round shape

Pathogenicity test

A pathogenicity test was conducted to test the ability of the pathogen to cause disease. Variation in the virulence among 20 isolates of *Rhizoctonia bataticola* was studied by their ability to infect and disease development on the soybean plants under greenhouse conditions. The different *R. bataticola* isolates showed wide variability in root rot infection of soybean plants ranging from 17.78 to 84.44 percent based on the percent Disease Incidence (PDI) as shown in Table 3. The differences in root rot infection caused by different isolates of *R. bataticola* were also observed

Grouping of isolates: 20 isolates of *Rhizoctonia bataticola* were grouped into five different categories based on their pathogenicity as shown in table 4. Three isolates *viz.*, Rb 2, Rb 5 and Rb 11 belonging to the highly pathogenic group showed > 70 per cent disease incidence, six isolates were from the strongly pathogenic group *viz.*, Rb 4, Rb7, Rb15, Rb18, Rb19, and Rb20 showed 51-70% per cent disease incidence and nine isolates from moderately pathogenic group *viz.*, Rb1, Rb3, Rb 8, Rb 9, Rb10, Rb 12, Rb13, Rb 16 and Rb 17 showed 21-50 per cent disease incidence. Two isolates were from weakly pathogenic *viz.*, Rb 6 and Rb 14 showed 1-20 per cent on pathogenicity. Significantly the higher pathogenicity (84.44) was shown by the isolate Rb5 over all other isolates studied. Present results were compared with Iqbal and Mukhtar (2014) who studied the pathogenic variability among the 65 isolates of *Macrophomina phaseolina* in which ten fungal isolates appeared least virulent, eight isolates appeared highly virulent against mungbean cultivars. The remaining isolates were regarded as moderately virulent. Gawade *et al.* (2017) conducted the pathogenicity of 20 isolates of soybean root rot caused by *Rhizoctonia bataticola*. Isolates showed variability between 48.00 and 69.33 per cent of the root rot infection of soybean. Similarly, Gade *et al.* (2018) [7] reported that pathogenic variability among 40 isolates of *R. bataticola*, causes root rot of soybean.

Table 1: Collection of diseased sample of root rot of soybean

Sr. No.	District	Tahsil	Location	Isolate code
1.	Kolhapur	Gadhinglaj	Mahagaon	Rb 1
2.	Sangli	Khanapur	Khanapur	Rb 2
3.	Satara	Satara	Vechale	Rb 3
4.	Ahmednagar	Rahata	Rahata	Rb 4
		Kopargaon	Kopargaon	Rb 5
			RanjangaonDeshmukh	Rb 6
		Sangamner	Nimon	Rb 7
5.	Nashik	Sinnar	Gonde	Rb 8
			Datli	Rb 9
		Niphad	Chandori	Rb 10
6.	Pune	Junnar	Aane	Rb 11
		Khed	Retavadi	Rb 12
7.	Solapur	Barshi	Kalegaon	Rb 13
		Mohol	Angar	Rb14
8.	Dhule	Dhule	Agriculture College Dhule	Rb 15
		Sakri	Pimpalner	Rb 16
			Samode	Rb 17
9.	Nandurbar	Shahada	Amode	Rb 18
		Navapur	Khandbara	Rb 19
10.	Jalgaon	Pachora	Pachora	Rb 20

Table 2: Morphological variability among different isolates of *Rhizoctonia bataticola*

Sr. No.	Isolates	Hyphal width (µm)	shape	Average size * of sclerotia (µm)
1	Rb 1	4.48	Oblong	57.41 × 42.34
2	Rb 2	5.44	Round	88.3 × 84.15
3	Rb 3	3.84	Oblong	62.15 × 40.30
4	Rb 4	3.85	Round	88.73 × 82.40
5	Rb 5	5.80	Round	103.88 ×98.2
6	Rb 6	3.20	Oblong	95.2 ×71.4
7	Rb 7	7.69	Oblong	63.10 × 45.25
8	Rb 8	3.84	Round	40.71×36.21
9	Rb 9	4.70	Oblong	73.45 × 57.65
10	Rb 10	4.41	Round	90.2 × 85.6
11	Rb 11	4.70	Oblong	110.24 × 84.11
12	Rb 12	5.21	Round	82 × 79
13	Rb 13	5.12	Round	44.75× 40.62
14	Rb 14	5.98	Round	66.85 × 63.95

15	Rb 15	6.41	Oblong	64.40 × 46.42
16	Rb 16	7.66	Round	79.11 × 77.86
17	Rb 17	2.56	Round	60.57 × 56.35
18	Rb 18	6.50	Round	68.35 × 63.20
19	Rb 19	6.41	Round	57.50 × 52.20
20	Rb 20	6.14	Oblong	78.51 × 52.29

*:Mean of ten observations / isolates

Table 3: Percent disease incidences (PDI) of isolates of *Rhizoctonia bataticola*

Sr. No.	Isolates	* PDI
1	Rb 1	42.22 (40.51)
2	Rb 2	77.78 (61.93)
3	Rb 3	33.33 (35.26)
4	Rb 4	53.33 (46.91)
5	Rb 5	84.44 (66.87)
6	Rb 6	20.00 (26.57)
7	Rb 7	62.22 (52.09)
8	Rb 8	44.44 (41.80)
9	Rb 9	46.67 (43.09)
10	Rb 10	37.78 (37.91)
11	Rb 11	71.11 (57.52)
12	Rb 12	48.89 (44.36)
13	Rb 13	44.44 (41.80)
14	Rb 14	17.78 (24.85)
15	Rb 15	66.67 (54.74)
16	Rb 16	26.67 (31.09)
17	Rb 17	31.11 (33.87)
18	Rb 18	55.56 (48.20)
19	Rb 19	64.44 (53.41)
20	Rb 20	57.78 (49.48)
21	Control	00.00
	SE	1.13
	CD at 5%	3.24

*Average of three replication PDI= Per cent Disease Incidence
Figures in parentheses are arcsine transformed values

Table 4: Grouping of *Rhizoctonia bataticola* isolates based on their pathogenic ability

Sr. No.	Category	Per cent (%) mortality due to root rot	No. of Isolates	Isolates
1.	Non Pathogenic	0	Nil	Nil
2.	Weakly Pathogenic	1-20	2	Rb 6, Rb 14
3.	Moderately pathogenic	21-50	9	Rb1, Rb 3, Rb 8, Rb9, Rb10, Rb 12, Rb 13, Rb 16, Rb 17,
4.	Strongly pathogenic	51-70	6	Rb 4, Rb 7, Rb 15, Rb 18, Rb 19, Rb20
5.	Highly pathogenic	>70	3	Rb 2, Rb 5, Rb 11

Conclusion

The present study investigates the variability of *R. bataticola* in major soybean-growing areas of Western Maharashtra. Root rot of soybean is distributed in all the districts of Western Maharashtra widely. The morphological and pathogenic variability was observed between the isolates. The hyphal width was ranged from 7.69 - 2.56 µm. According to shape isolates were grouped into round and oblong. Based on micrometry, the average size of sclerotia ranged between 110.24 x 84.11 - 40.71 x 36.21 µm. In pathogenicity within all twenty isolates, three isolates were found most virulent and only two isolates showed least virulent whereas nine isolates showed moderate Virulent and six isolates showed Strongly Virulent.

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