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Pathogenic Variability of Soybean Charcoal Rot Caused *Macrophomina phaseolina* Isolates against Pulse Crops

Ashwini Kumar[1], M. Surya Prakash Reddy[2*], Anupam Srivastava[3] and Varma R. K.[4]

[2, 3,4]Department of Plant Pathology, JNKVV, Jabalpur-M.P-482004

[1]Department of Plant Pathology, B.M College of Agriculture, RVSKVV, Khandwa, M.P.

*Corresponding Author E-mail: suryapath017@gmail.com

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ABSTRACT

Macrophomina phaseolina is the most devastating, emerging and root killer pathogen which causes charcoal rot and root rot diseases in various economically important crops. Macrophomina phaseolina 16 isolates of soybean collected from various districts of Madhya Pradesh state. Pathogenic variability among the 16 isolates of Macrophomina phaseolina to test line of susceptible varieties of different host crops as soybean, chick pea, mungbean, urdbean and cowpea, were used to check wide range host. The parameters can be observed from experiment Root Index, Mortality, Root Mortality Per unit, Avirulent Virulent, higly Virulent. To avoid crop rotations of soy bean with any pulse crop for future.

Keywords: *Soybean isolates, Macrophomina phaseolina, Pathogenic Variability*

INTRODUCTION

Macrophomina phaseolina, the causal agent of charcoal rot is a soil and seed-borne polyphagous pathogen. It causes diseases of more than 500 crop and non-crop species, including economically important hosts such as soybean, common bean, corn, sorghum, cowpea, peanut and cotton Dhingra and Sinclair (1977), Ndiaye et al. (2010). The fungus has a worldwide distribution, but is regarded as economically more important in subtropical and tropical countries with semi-arid climates (Wrather, et al., 1997, Wrather, et al., 2001). *Macrophomina phaseolina* induces diseases on a range of crops, ranging from seedling blight, root and stem rot, wilt, and pre- to post-emergent damping off, which result in decreased stem height, girth, root and head weight, or death, of affected plants. The abundant production of minute black sclerotia of the fungus cause the rotted tissues to become blackened, and for this reason the various diseases are known as charcoal rot. Different isolates of *R. bataticola* obtained from different plant species, and plant parts of the same host showed variability Prameela and Singh, (1998) Meena et al. (2006). Sixty four isolates of *M. phaseolina* from sunflower Manici et al. (1992) and cotton Monga et al. (2004) fell into 3 groups viz., highly virulent, virulent and poorly virulent, of pathogenic variability. In India 70 percent loss cause by charcoal rot has been reported.

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The disease is distributed in Madhya Pradesh, Maharashtra, Rajasthan and Delhi. The important host is soybean, sunflower, safflower sesame, sorghum, rice, green-gram, black-gram, cowpea, pigeon-pea and potato. In M.P. the crop sequence gram – soybean and dry spell during pod filling have played major role in enhancing the incidence of charcoal rot of soybean caused by *Macrophomina phaseolina*. Therefore the present investigation is under taken with the following.

MATERIALS AND METHODS

Seeds

Seeds of different crops were used for study. Seed of soybean cultivar JS 95-60, Chickpea (JG 212) which were obtained from All India Co-ordinated Research Project on soybean, mungbean (Ganga-8), and urid bean (T-9) from Agronomy Department. Cowpea (Mayur) from Horticulture Department College of Agriculture Jabalpur. Seeds were sterilized in 0.1% of $HgCl_2$ solution for 2 minute followed by three changes in sterilized distilled water to remove trace of $HgCl_2$.

Seed germination test paper

Seed germination test paper. (45X30 cm) were purchased from Ajay Kumar and Sons, New Delhi.

Test pathogen

Macrophomina phaseolina (Rhizoctonia bataticola) the causal organism of charcoal rot of soybean.

Disease samples of soybean collected from various districts of Madhya Pradesh state.

District	Locality of collocation of isolates	Designation of isolates of *Macrophomina phaseolina*
Jabalpur	Adhartal	I_1
Vidisha (Ganjbasoda)	K.V.K Farmer field	I_2 I_3
Narsingpur	K.V.K	I_4
Sagar	K.V.K Farmer field	I_5 I_6
Narsingpur	Farmer field	I_7
Jabalpur	Farmer field	I_8
Narsinghpur (Gadarwara)	Farmer field	I_9
Jabalpur	Khamariya Krishi Nagar Farm Krishi Nagar Farm	I_{10} I_{11} I_{12}
Indore	K.V.K.	I_{13}
Sagar (Garhakota)	Farmer field	I_{14}
Rewa	K.V.K	I_{15}
Tikamgarh	K.V.K	I_{16}

Pathogenic variability

Pathogenicity test

Blotter paper technique as described by Nene et al. (1981), for *Rhizoctonia bataticola. (Macrophomina phaseolina).*To conduct the pathogenicity test of soybean (JS 95- 60), chick pea (JG 212), mungbean (Ganga-8), urdbean (T-9), and cowpea (Mayur), 20 surface sterilized (35 second in 2.5% sodium hypo-chloride) seeds of the test lines in autoclaved riverbed sand placed in 15-cm pots. Approximately 1 kg of sand will be required for one pot. Nurse the seedlings till these are 5 days old from Prepare potato-dextrose broth. Place 100 ml broth in one 250-ml flask and prepare as many flasks as needed. Autoclave at 15 Ib for 20 min. For testing ten lines at one time one

flask of inoculum will be needed. Inoculate the medium with the fungus. Incubate for 5 days at 25°C. Remove the mycelial mats from the flasks at the end of the incubation period. Add two mycelial mats to 100 ml sterilized distilled water and macerate these in a Warming blender for 1 min (operate the blender intermittently). Place this inoculum in a beaker of a suitable size. Uproot the 5-day-old seedlings of the test lines (step 2). Wash the root system in running water and rinse in sterilized distilled water. Hold all seedlings of a test line in a hand and dip the roots of these in the inoculum with an up and down movement for about 30 seconds. Remove excess inoculum by touching the edge of the beaker. Place 20 seedlings of the test line side by side on a blotter paper (size 45 cm X 25 cm with one fold; any collar; thin) so that only the cotyledons and roots are covered and the green tops of the seedlings remain outside the blotter paper after it is folded. Fold the blotter paper and moisten it adequately but not excessively. One folded blotter paper will have seedlings of one test line. Inoculate seedlings of a susceptible check with each batch of test seedlings. Keep folded blotters, one on top of the other, in heaps of ten in a tray. One of these ten blotters should have the seedlings of the susceptible check. Place the trays in an incubator at 35°C for 8 days. Provide 12-hr artificial light. Moisten the blotters adequately every day. At the end of the incubation period (8 days), examine the seedlings for the extent of root damage, and score for the disease. The fungus survives on infected plant debris and is also transmitted through seed. The disease spreads rapidly under cool, wet, and windy conditions.

Root mortality (%) per unit:

Charcoal rot root mortality (%) per unit was calculated by the following formula.

$$\text{Root mortality (\%) per unit} = \frac{\text{Mortality (\%)}}{\text{Root index (\%)}}$$

Percent category of charcoal rot of soybean

S. No.	Category	Charcoal rot %
1	Avirulent	<10
2	Virulent	10-50
3	Highly virulent	>50

RESULTS AND DISCUSSION

Table 1: Reaction of sixteen isolates of *Macrophomina phaseolina* causing charcoal rot of soybean, against soybean JS 95-60.

Isolate No.	Root Index (%)	Mortality (%)	Root Mortality % Per unit	Category
1	50.0	94.1	1.9	HV
2	48.4	100	2.1	HV
3	62.9	100	1.6	HV
4	58.8	94.4	1.6	HV
5	26.6	77.7	2.9	HV
6	24.1	82.3	3.4	HV
7	43.6	94.4	2.2	HV
8	45.2	88.2	2.0	HV
9	60.6	100	1.7	HV
10	52.9	94.1	1.8	HV
11	39.4	100	2.5	HV
12	47.6	88.2	1.9	HV
13	34.1	82.3	2.4	HV
14	45.0	81.2	1.8	HV
15	44.4	94.4	2.1	HV
16	40.0	100	2.5	HV

Table 2: Reaction of sixteen isolates of *Macrophomina phaseolina* causing charcoal rot of soybean, against chickpea JG 212.

Isolate No.	Root Index (%)	Mortality (%)	Root Mortality % Per unit	Category of pathogen
1	13.6	90.9	6.7	HV
2	38.5	100	2.6	HV
3	26.9	92.3	3.4	HV
4	18.5	92.8	5.0	HV
5	32.8	92.8	2.8	HV
6	34.4	100	2.9	HV
7	06.6	58.3	8.8	HV
8	10.0	61.5	6.2	HV
9	05.9	76.9	8.3	HV
10	27.6	100	3.6	HV
11	05.9	69.2	9.5	HV
12	16.4	100	6.1	HV
13	25.3	80	3.2	HV
14	05.5	66.6	6.2	HV
15	16.3	72.7	4.5	HV
16	26.8	87.5	3.3	HV

Table 3: Reaction of sixteen isolates of *Macrophomina phaseolina* causing charcoal rot of soybean, against mung bean Ganga – 8

Isolate No.	Root Index (%)	Mortality (%)	Root Mortality % Per unit	Category of pathogen
1	11.2	55.0	4.9	HV
2	13.0	60.0	5.0	HV
3	19.2	63.1	3.2	HV
4	5.6	55.5	9.9	HV
5	12.7	52.3	4.1	HV
6	15.8	84.2	5.3	HV
7	13.5	65.0	4.8	HV
8	5.9	55.0	9.3	HV
9	17.8	63.1	3.5	HV
10	9.0	68.7	7.6	HV
11	7.4	55.5	7.5	HV
12	15.5	55.5	3.5	HV
13	7,3	50.0	6.8	V
14	14.4	50.0	3.4	V
15	13.9	61.9	4.4	HV
16	7.3	38.0	5.2	V

Table 4: Reaction of sixteen isolates of *Macrophomina phaseolina* causing charcoal rot of soybean, against urdbean T-9

Isolate No.	Root Index (%)	Mortality (%)	Root Mortality % Per unit	Category of pathogen
1	24.8	44.0	1.7	V
2	17.6	52.6	2.9	HV
3	14.0	40.9	2.9	V
4	13.5	42.8	3.1	V
5	8.1	36.8	4.5	V
6	4.5	35.0	7.7	V
7	19.7	42.1	2.1	V
8	6.7	30.0	4.4	V
9	15.2	47.6	3.1	V
10	9.1	42.8	4.7	V
11	14.0	40.9	2.9	V
12	18.0	43.4	2.4	V
13	11.1	41.1	3.7	V
14	11.6	47.0	4.0	V
15	8.9	43.4	4.8	V
16	8.0	40.0	5.0	V

Table 5: Reaction of sixteen isolates of *Macrophomina phaseolina* causing charcoal rot of soybean, against against cowpea Mayur

Isolate No.	Root Index (%)	Mortality (%)	Root Mortality % Per unit	Category of pathogen
1	6.4	35.2	5.5	V
2	18.8	61.1	3.3	HV
3	14.3	60.0	4.2	HV
4	6.8	68.7	10.1	HV
5	7.3	53.3	7.3	HV
6	8.0	44.4	5.6	V
7	16.5	68.7	4.2	HV
8	16.5	78.5	4.8	HV
9	6.5	56.2	8.6	HV
10	10.6	56.2	5.3	HV
11	9.5	56.2	5.9	HV
12	11.0	50.0	4.5	V
13	12.6	52.9	4.2	V
14	7.0	47.0	6.7	V
15	7.1	43.7	6.2	V
16	6.5	43.7	6.7	V

Table 6: Categorization of *Macrophomina phaseolina* on the basis of virulence

Isolates No.	Soybean	Chickpea	Mungbean	Urdbean	cowpea
1.	HV	HV	HV	V	V
2.	HV	HV	HV	HV	HV
3.	HV	HV	HV	V	HV
4.	HV	HV	HV	V	HV
5.	HV	HV	HV	V	HV
6.	HV	HV	HV	V	V
7.	HV	HV	HV	V	HV
8.	HV	HV	HV	V	HV
9.	HV	HV	HV	V	HV
10.	HV	HV	HV	V	HV
11.	HV	HV	HV	V	HV
12.	HV	HV	HV	V	V
13.	HV	HV	V	V	V
14.	HV	HV	V	V	V
15.	HV	HV	HV	V	V
16.	HV	HV	V	V	V

Reaction of different soybean isolates of *Macrophomina phaseolina* causing Charcol rot of soybean, against soybean JS 95 – 60 (Table 1).

Data presented in **Table 1** clearly indicated that Root index (%) ranged from 24.1 to 62.9 %. The minimum root index (24.1%) was recorded in isolates I_6 (Sagar, Farmer field) and I_5 (Sagar, KVK) with 26.6 per cent. Maximum and above 50 per cent Root index (%) was observed in 5 isolates i.e. I_3 (Ganjbasoda, Farmer field), I_9 (Gadarwada, Farmer field), I_4 (Narsinghpur, KVK), I_{10} (Jabalpur, Khamariya) and I_1 (Jabalpur, Adhartal).Observation on mortality percentage it was recorded that it ranged from 77.7 to 100.0 per cent. All the 16 isolates were found to cause mortality more than 77.7%.Cent Per cent mortality was recorded in isolates I_2 (Gangbasoda, KVK), I_3 (Gangbasoda, Farmer field) I_9 (Gadarwada, Farmer field), I_{11} (Jabalpur, Krishi nagar farm) and I_{16} (Tikamgrah, KVK).Data observed on mortality per unit showed the range of 1.6 to 3.4. Maximum mortality per unit of 3.4 and 2.9 was recorded in isolates I_6 (Sagar, Farmer field) and I_5 (Sagar, KVK) respectively. All the 16 isolates had shown highly virulent (HV) reaction to soybean c.v. JS 95-60.

Reaction of sixteen isolates of *Macrophomina phaseolina,* causing Charcoal rot of soybean, against chickpea JG 212(Table 2).

The data presented in **Table 2** exhibited that root index percentage of chickpea inoculated with 16 isolates ranged from 6.6 to 38.5%. Minimum Root index (%) of 6.6 and 7.3 per cent was recorded in I_7 (Narsinghpur, Farmer field) and I_{11} (Jabalpur, Krishi nagar farm) respectively. Maximum of 38.5, 34.4 and 32.8 was observed in I_2 (Ganjbasoda, KVK), I_6 (Sagar, Farmer field) and I_5 (Sagar, KVK) respectively. Other isolates had root index (%) were between 10.0 to 27.6 per cent. Per cent mortality ranged from 58.3 to 100.0 per cent four isolates i.e. I_2 (Ganjbasoda, KVK), I_6 (Sagar, Farmer field), I_{10} (Jabalpur, Khamariya) and I_{12} (Jabalpur, Krishi nagar farm) had 100% mortality, out of 16 only one isolates I_7 (Narsinghpur, Farmer field) has mortality less than 60 per cent. Data calculated for causing seedling mortality per unit root index showed variation from 2.6 to 9.5. Isolates I_2 (Ganjbasoda, KVK), I_5 (Sagar, KVK) and I_6 (Sagar, Farmer field) had minimum mortality per unit i.e. 2.6, 2.8 and 2.9. Three isolates I_{11} (Jabalpur, Krishi nagar farm), I_7 (Narsinghpur, Farmer field) and I_9 (Gadarwada, Farmer field) had shown maximum mortality per unit of 9.5, 8.8 and 8.3 respectively. All the 16 isolates had shown highly virulent (HV) reaction to chickpea C.V. JG 212 under the investigation.

Reaction of sixteen isolates of *Macrophomina phaseolina,* causing Charcoal rot of soybean, against mungbean Ganga – 8(Table 3).

Data on reaction of 16 isolates on mungbean **Table 3** showed that root index per cent varied from 5.6 to 19.2 per cent. Minimum of 5.6 percent and 5.9 per cent was recorded in I_4 (Narsinghpur, KVK) and I_8 (Jabalpur, Farmer field) respectively. Highest of 19.2 per cent was recorded in I_3 (Ganjbasoda, Farmer field) followed by 17.8 in I_9 (Gadarwada, Farmer field). Other isolates ranged between 8.0 to 15.8.Observation on per cent seedling mortality ranged from 38.0 to 84.2 per cent. Out of 16 only 3 isolates I_{13} (Indore, KVK), I_{14} (Garakhota, Farmer field) and I_{16} (Tikamgarh, KVK) could cause seedling mortality below 50 per cent. Other isolates had more than 50 per cent but none of them caused 100 per cent mortality. Mortality per unit area ranged from 3.3 to 9.9 per cent. Maximum mortality per unit of 9.9 and 9.3 was recorded in I_4 (Narsinghpur, KVK) and I_8 (Jabalpur, Farmer field) respectively. Minimum of 3.3 was noted in I_3 (Ganjbasoda, Farmer field) followed by 3.5 in I_9 (Gadarwada, Farmer field) and I_{14} (Garakhota, Farmer field).Out of 16, 13 isolates were designated as highly virulent (HV) 3 as virulent (V) none of them were found in avirulent category.

Reaction of sixteen isolates of *Macrophomina phaseolina* causing Charcoal rot of soybean, against urdbean T-9 (Table 4).

The data exhibited in Table 4 showed wide variation among 16 isolates as for as the root index per cent was concerned. It varied from 4.5 to 24.8 per cent. Minimum root index per cent of 4.5 and 6.7 per cent was noted in I_6 (Sagar, Farmer field) and I_8 (Jabalpur, Farmer field) whereas 24.8 per cent was recorded in I_1 (Jabalpur, Adhartal) followed by 19.7 in I_7 (Narsinghpur, Farmer field).Range of per cent seedling mortality was quite narrow and between 30.0 to 52.6.Maximum mortality of 52.6 and 47.6 per cent was noted is I_2 (Ganjbasoda, KVK) and I_9 (Gadarwada, Farmer field) respectively. Mortality more than 60 per cent was not recorded in any of the isolates.Mortality per unit varied between 1.8 to 7.8.Highest mortality per unit of 7.8 was observed in I_6 (Sagar, Farmer field) followed by 5.0 is I_{16} (Tikamgarh, KVK).Data clearly indicated that only one isolate I_2 (Ganjbasoda, KVK) fell in highly virulent category and rest of the 15 isolates in virulent category. None of the isolate was fell in avirulent category.

Reaction of sixteen isolates of *Macrophomina phaseolina* causing Charcoal rot of soybean, against cowpea Mayur (Table 5).

Data recorded in **Table 5** on reaction of 16 isolates on cowpea indicated that root index

percentage varied from 6.4 to 18.8%. Minimum root index (%) of 6.4 and 6.5 was recorded in I_1 (Jabalpur, Adhartal) and I_9 (Gadarwara, Farmer field). Maximum of 18.8 per cent was observed in I_2 (Ganjbasoda, KVK) followed by 16.5 per cent by isolate I_8 (Jabalpur, Farmer field).Seedling mortality ranged from 35.2 per cent I_1 (Jabalpur, Adhartal) to 78.5 per cent I_8 (Jabalpur Farmer field).Only four isolates i.e. I_2 (Ganjbasoda, KVK), I_4 (Narsingpur, KVK), I_7 (Narsingpur, Farmer field) and I_8 (Jabalpur, Farmer field) caused mortality more than 60 per cent. Data on mortality per unit varied from 3.3 to 10.1 per cent. Minimum of 3.3 per cent mortality per unit was recorded in I_2 (Ganjbasoda, KVK) followed by 4.2 per cent by I_2 (Ganjbasoda, KVK), I_7 (Narsingpur, Farmer field) and I_{13} (Indore, KVK). Highest mortality per unit was recorded in isolate I_4 (Narsingpur, KVK) followed by 8.6 by I_9 (Gadarwara, Farmer field). Reaction of 16 isolates based on seedling mortality showed 9 isolate as highly virulent, 7 as virulent. None of the isolates had shown avirulent reaction in cowpea.

Saleh et al. (2010), evaluated the degree of populations of *M. phaseolina* by comparing 143 isolates from maize, sorghum, soybean fields and from eight plant species of tall grass prairie. Purkayastha et al. (2006) reported by variability in morphology, physiology, genetics, pathogenicity and so forth is imperative for the fungus to have better adaptation in response to diversified environmental conditions. It also leads to host plant resistance, development of resistant varieties of different crops against disease and implementation of new disease controlling strategies. Purkayastha et al. (2004), also found relationship between morphological variations and pathogenicity. Mihail and Taylor (1995) suggested that, due to heterogenic nature of *M. phaseolina*, categorization into distinct subgroups based upon pathogenicity and morphology could not take place.

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