



## Charcoal Rot and Yellow Mosaic Virus Diseases of Soybean Under Hot Spot Condition: Symptoms, Incidence and Resistance Characterization

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### Abstract

Charcoal rot (CR) and Yellow mosaic virus (YMV) caused by *Macrophomina phaseolina* (Tassi) Goid and *Mungbean Yellow Mosaic India Virus* (MYMIV), respectively are important soybean diseases in India. In a field experiment, forty one promising soybean genotypes across the country plus four check namely JS 20-29, JS 93-05, JS 95-60 and JS 335 were observed throughout the season (Kharif -2017) for different aspect of diseases including resistance evaluation. Incidence of YMV was higher in early stage and started declining after 60 days of crop. Whereas charcoal rot was very severe in later stage of crop and maximum plant mortality occurred in reproductive stages (60-75 days after sowing). Conspicuously, contrast yellow green patches (mottles) on the leaves in YMV and blackening on or below the epidermis of lower stem and root of infected dead plants in CR were major characteristic symptoms of respective disease, respectively. Out of forty one, only nine entries i.e. AUKS 174, JS 21-17, NRC 133, AMS 100-39, RVS 2011-1, RSC 11-07, PS 1611, RSC 11-03 and SKF-SPS-11 were found to be absolute resistant against CR. Whereas forty nine percent entries were exhibited susceptibility to highly susceptibility against CR with highest plant mortality of 86.5 per cent in JS 95-60. In case of YMV, thirty one entries were found to be highly resistant and highest coefficient of infection (22.60) was found in JS 93-05. Altogether, only six entries namely JS 21-17, AMS 100-39, RVS 2011-1, PS 1611, RSC 11-03 and SKF-SPS-11 were found to be absolutely/highly resistant against both the diseases (CR plus YMV).

**Keywords:** Soybean, Charcoal rot, YMV, Symptoms, incidence, Resistance

### 1. Introduction

Soybean (*Glycine max* (L.) Merrill) is an important oil seed crop grows across the world. Soybean is also a seed legume which contributes to 25 % of the global edible oil and about two-thirds of the world's protein concentrate for livestock feeding (Agarwal et al., 2013). In India, it is major Kharif crop and presently cultivated in the area of 10.97 million ha area with total production of 10.99 million tons and productivity of 1002 kg/ha<sup>-1</sup> during 2016-17. Among different states, Madhya Pradesh occupies largest area of 54.01 lakh ha with average productivity of 1020 kg/ha and total production is 55.06 lakh ton during 2016-17 (Anonymous, 2018). Soybean is subjected to many diseases caused by fungi, bacteria, viruses, mycoplasma and nematodes (Sweets, 2008). In Madhya Pradesh, Charcoal rot and Yellow Mosaic virus disease has become the serious challenge in soybean cultivation. Charcoal rot caused by *Macrophomina*

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*phaseolina* (Tassi) Goid is the second most important yield reducing disease after brown spot (*Septoria glycines*) in the soybean growing countries across the world (Wrather et al., 2001). The pathogen of soybean charcoal rot (*Macrophomina phaseolina*=*Rhizoctonia bataticola*) also causes seedling blight, root rot and root and stem rot in many other cultivated crops as Common bean, Sorghum, Maize, Cotton, Peanut, Cowpea (Dhingra and Sinclair, 1977; Diourte et al., 1995). Pathogen of CR, *Macrophomina Phaseolina* infects vascular system and interferes with the normal plant function of transporting water and nutrients that resulted in wilting and premature leaf death of plant (Khan, 2007). Infection of charcoal occurs through the cropping season, it can infect seedlings to mature plants (Luna et al., 2017). Charcoal rot causes plant death during reproductive stages of crop and the disease is characterized by presence of small black microsclerotia in vascular tissues and on lower part of plant (Bradley and Rio, 2003). In central India, Yellow mosaic disease of soybean and blackgram is caused by Mungbean Yellow Mosaic India Virus (MYMIV) (Usharani et al., 2004; Girish and Usha, 2005). MYMIV is single stranded DNA virus of geminiviridae family (Kumar et al., 2014). The yellow mosaic virus (YMV) disease caused by begomovirus (geminiviridae family) is transmitted by whitefly *Bemisia tabaci* which also infect other legumes such as blackgram, greengram etc (Nene, 1969; Govindan et al., 2014). In a recent study, YMV can cause as high as 85.7% yield reduction, if the plants affected severely in earlier stage of crop (Amrate et al., 2020). Charcoal rot and YMV diseases are very difficult to manage in field conditions and development of resistant varieties against the prevalent races/strains of the pathogen are major management strategy. Keeping this in mind, the experiment was undertaken to identify resistance sources against both the diseases and to reveal other aspects of diseases under high disease pressure conditions. The findings could be useful in developing integrated disease management practices and resistant varieties for combating the diseases in upcoming time.

## 2. Materials and Methods

### 2.1. Experimental site and details

A field experiment with forty one promising soybean genotypes across the country plus four check namely JS 20-29, JS 93-05, JS 95-60 and JS 335 were conducted to observe different aspect and resistance characterization for Charcoal rot (CR) and Yellow mosaic virus (YMV) in the research field of All India Coordinated Research Project (AICRP) on Soybean at J.N.K.V.V., Jabalpur centre. This centre has identified as hot spot for CR and YMV both the disease by AICRP on Soybean. The sowing of 41 entries plus 4 checks (JS 20-29, JS 93-05, JS 95-60 and JS 335) in non-replicated manner was done in the last week of June, 2017. Observation for the time of appearance of disease and progress (incidence and severity of CR and YMV) were taken at fifteen days interval during the entire cropping season. The per cent disease mortality for CR and incidence and coefficient of infection (CI) for YMV were calculated as per scale given below.

### 2.2. Charcoal rot resistance characterization

Genotypes were assigned resistance as the scale give below

Rating	Descriptions	Categories
0	No mortality	Absolutely resistant (AR)
1	1 % mortality	Highly resistant (HR)
3	1.1 to 10% mortality	Moderately resistant (MR)
5	10.1 to 25% mortality	Moderately susceptible (MS)
7	25.1 to 50% mortality	Susceptible (S)
9	More than 50% mortality	Highly susceptible (HS)

### 2.3. YMV resistance characterization

It is based on per cent incidence (number of plant infected) and their interaction with per cent disease severity (number of leaves having symptom over total number of leaves in a single plant and averaged from 10 such plants). Symptom severity grades (0-4) and response value (0-1) were assigned to different entries according disease severity and the coefficient of infection (CI) was calculated by multiplying the percent disease incidence to the response value assigned for each severity grade following standard methodology (Singh and Singh, 2000).

Symptoms	Severity grade	Response value	Coef- ficient of infection (CI)	Disease re- action
Symptoms absent	0	0	0-4	Highly resistant (HR)
Very mild symptoms upto 25% leaves	1	0.25	5-9	Resistant (R)
Appearance of symptoms in 26-50% leaves	2	0.50	10-19	Moderately resistant (MR)
Appearance of symptoms in 51-75% leaves	3	0.75	20-39	Moderately susceptible (MS)
Severe disease infection in symptoms (>75% leaves)	4	1.00	40-69	Susceptible (S)
			70-100	Highly susceptible (HS)

The results of study has been summarized and presented in Table 1 and 2.



Table 1: Severity score and resistant reaction of promising entries against Charcoal rot and YMV diseases during *kharif* 2017-18

Entries	Per cent Mortality and Coefficient of infection						Entries	Per cent Mortality and Coefficient of infection						
	CR			YMV				CR			YMV			
	% Mor- tality	Scale	Reac- tion	CI	Scale	Reac- tion		% Mor- tality	Scale	Reac- tion	CI	Scale	Reac- tion	
NRC 128	80.5	9	HS	0.0	0-4	HR	AMS 100-39	0.0	0	AR	0.0	0-4	HR	
RVS 2011-3	2.3	3	MR	2.25	0-4	HR		NRC 136	15.6	5	MS	0.0	0-4	HR
DSb 34	56.7	9	HS	0.0	0-4	HR		RVS 2011-1	0.0	0	AR	0.0	0-4	HR
MAUS 725	64.5	9	HS	5.5	5-9	R	CSB 10112	70.5	9	HS	0.0	0-4	HR	
SL 1068	58.6	9	HS	0.0	0-4	HR	PS 1613	45.6	7	S	0.0	0-4	HR	
JS 21-15	25.6	7	S	0.0	0-4	HR	NRC 131	26.5	7	S	1.25	0-4	HR	
AUKS 174	0.0	0	AR	10.5	10-19	MR	KDS 992	8.5	3	MR	2.4	0-4	HR	
NRC 137	16.5	5	MS	0.0	0-4	HR	RSC 11-07	0.0	0	AR	13.5	10-19	MR	
VLS 95	55.4	9	HS	0.0	0-4	HR	NRCSL 1	45.6	7	S	0.0	0-4	HR	
CSB 10084	72.6	9	HS	5.25	5-9	R	PS 1611	0.0	0	AR	0.0	0-4	HR	
MACS 1493	4.8	3	MR	11.35	10-19	MR	RSC 11-03	0.0	0	AR	0.0	0-4	HR	
JS 21-17	0.0	0	AR	0.0	0-4	HR	NRC 134	16.5	5	MS	3.20	0-4	HR	
NRC 130	12.6	5	MS	3.25	0-4	HR	NRC 129	3.5	3	MR	0.0	0-4	HR	
TS 53	80.5	9	HS	2.50	0-4	HR	RVS 2011-2	28.6	7	S	0.0	0-4	HR	
SL 1123	45.5	7	S	5.50	5-9	R		SKF-SPS-11	0.0	0	AR	0.0	0-4	HR
BAUS 102	0.8	1	HR	11.50	10-19	MR		SKF-1050	40.5	7	S	0.0	0-4	HR
MACS 1575	75.8	9	HS	0.0	0-4	HR	Shalimar Soybean	62.8	9	HS	0.0	0-4	HR	
MAUS 731	2.0	3	MR	3.30	0-4	HR	JS 20-29 (c)	69.4	9	HS	2.75	0-4	HR	
NRC 132	4.6	3	MR	1.25	0-4	HR	JS 95-60 (c)	86.5	9	HS	8.4	5-9	R	
VLS 94	5.8	3	MR	10.5	10-19	MR	JS 93-05 (c)	45.5	7	S	22.60	20-39	MS	
AMS 2014-1	0.5	1	HR	12.5	10-19	MR	JS 335 (c)	23.7	5	MS	18.5	10-19	MR	
KDS 1095	33.5	7	S	0.0	0-4	HR								
NRC 133	0.0	0	AR	14.25	10-19	MR								
DS 3108	52.7	9	HS	0.0	0-4	HR								

Table 2: Appearance and progress of charcoal rot and YMV in respect to plant age during *kharif*, 2017-18

Disease*	First symptoms appearance		Disease progress at days after sowing				
	Month	DAS	30	45	60	75	90
YMV (% Incidence)	Second week of July	16	17.36	30.56	45.68	55.10	60.38
Charcoal rot (% mortality)	Third week of August	47	0.0	0.0	11.88	52.04	79.18

\*Average of highly infected five genotypes; DAS: Days after sowing

### 3. Results and Discussion

#### 3.1. Time of appearance, symptoms and progress of disease

##### 3.1.1. Charcoal rot

##### 3.1.1.1. Symptoms

Initially, the leaves of infected plant became dull greenish to yellowish and plants started wilting and dying within 4-10 days. Leaves of the infected/dead plants remained attached



and epidermis of lower stem appeared grey to black. Removing of epidermis of lower stem and root numerous small, black bodies (microsclerotia) are visible which gives charcoal like blackish look to tissue. Greyish to blackish appearance of internal tissue was also seen upon breaking and splitting of the roots and lower stem (Figure 1).



Figure 1: Characterization of Charcoal rot: Grayish black appearance of lower stem (a), blackening and shredding on infected root and lower stem and presence of microsclerotia in xylem vessels (b), leaves remain attached on dead plant (c) and yellowing and wilting of genotypes in field (d), respectively

### 3.1.1.2. Disease progress

Symptoms of charcoal rot was first noticed on JS 95-60 during third week of august. In all the entries, overall mortality due to CR was varied from 0.0 to 86.5%. In highly infected genotypes (five), the per cent mortality was recorded 11.88, 52.04 and 79.18 at 60, 75 and 90 days after sowing of crop, respectively. The incidence of charcoal rot in all the genotypes was noticed in reproductive stages of crop (Table 2 and Figure 2 and 3).

### 3.1.2. Yellow mosaic virus

#### 3.1.1.1. Symptoms

Varying size yellow mottles or spots were first appeared on young leaves. As severity progressed, the leaves which infected earlier turned almost yellow but the major veins remain green. Contrast yellow green patches (mottles) on the leaves were major conspicuous symptoms on all the infected line. Whereas, rusty necrotic spots were also seen in highly infected leaves (Figure 4).

### 3.1.1.2. Disease progress

YMV was first appeared just after 15 days of sowing during

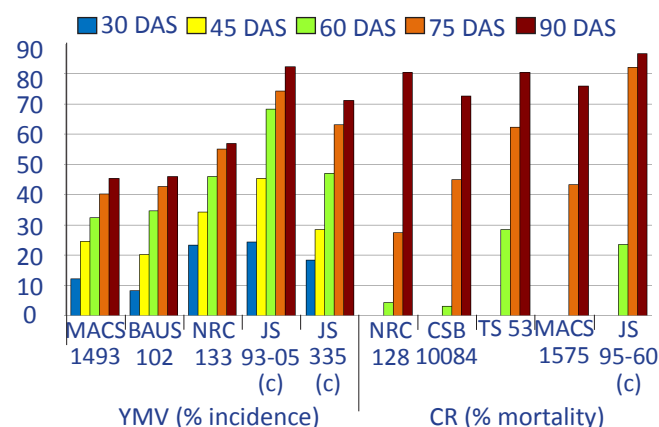


Figure 2: Fifteen days interval progress of YMV and Charcoal rot in highly infected genotypes

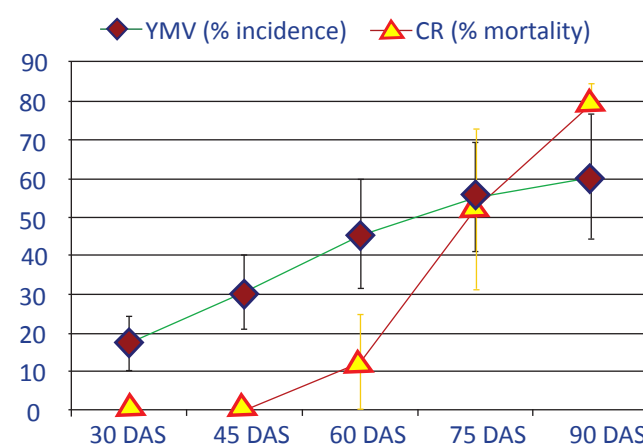


Figure 3: Average trend (fifteen days interval) of YMV and Charcoal rot progress in highly infected genotypes

second week of July in the varieties JS 335 and JS 93-05. However, the incidence of YMV has noticed in many genotypes till the end of season but intensity of disease was not severe. The average incidence of five highly infected genotypes was 17.36, 30.56, 45.68, 55.10 and 60.38% at 30, 45, 60, 75 and 90 days after sowing of crop, respectively. Rate of incidence increase was higher in early stage of crop and it declined after 60 days crop (Table 2 and Figure 2 and 3).

### 3.2. Resistance characterization

#### 3.2.1. Charcoal rot

Out of forty one, only nine i.e. AUKS 174, JS 21-17, NRC 133, AMS 100-39, RVS 2011-1, RSC 11-07, PS 1611, RSC 11-03 and SKF-SPS-11 were found to be free from CR infection and categorized as absolute resistant. Whereas in two entries namely BAUS 102 and AMS 2014-1 disease appeared in traces categorized as highly resistant. Seven entries showed moderate resistant while others were found to be moderate susceptible to highly susceptible against CR. Out of total tested (including checks), 49.0% genotypes were reacted as susceptible to highly susceptible (Table 1 and Figure 5).

#### 3.2.2. Yellow mosaic virus

Thirty one entries namely NRC 128, RVS 2011-3, DSb 34, SL





Figure 4: Characterization of YMV: Yellow spots appearance (a), plant showing yellow spot with initially infected leaves became almost yellow except veins (b) and contrast yellow and green patches on leaves with earlier infected leaves showing some necrotic spots (c), respectively.

1068, JS 21-15, NRC 137, VLS 95, JS 21-17, NRC 130, TS 53, MACS 1575, MAUS 731, NRC 132, KDS 1095, DS 3108, AMS 100-39, NRC 136, RVS 2011-1, CSB 10112, PS 1613, NRC 131, KDS 992, NRCSL 1, PS 1611, RSC 11-03, NRC 134, NRC 129, RVS 2011-2, SKF-SPS-11, SKF-1050 and Shalimar Soybean were found to be highly resistant as coefficient of infection was either zero or up to 4. Other entries were found to be reacted as resistant and moderate resistant. Only check variety JS 93-05 was found to be moderately susceptible (Table 1 and Figure 5).

### 3.2.3. CR plus YMV resistant

While considering the infection of both pathogen only six entries namely JS 21-17, AMS 100-39, RVS 2011-1, PS 1611, RSC 11-03 and SKF-SPS-11 were recorded no incidence and categorized as absolutely/highly resistant against both Charcoal rot and YMV disease.

Earlier study also confirmed resistance and susceptibility in soybean genotypes for both Charcoal rot and YMV. Ansari (2007) evaluated different elite soybean genotypes against charcoal rot under field conditions in Madhya Pradesh, India and found genotypes in different categories as highly resistant (1.0% mortality), moderately resistant (1.1-10.0%

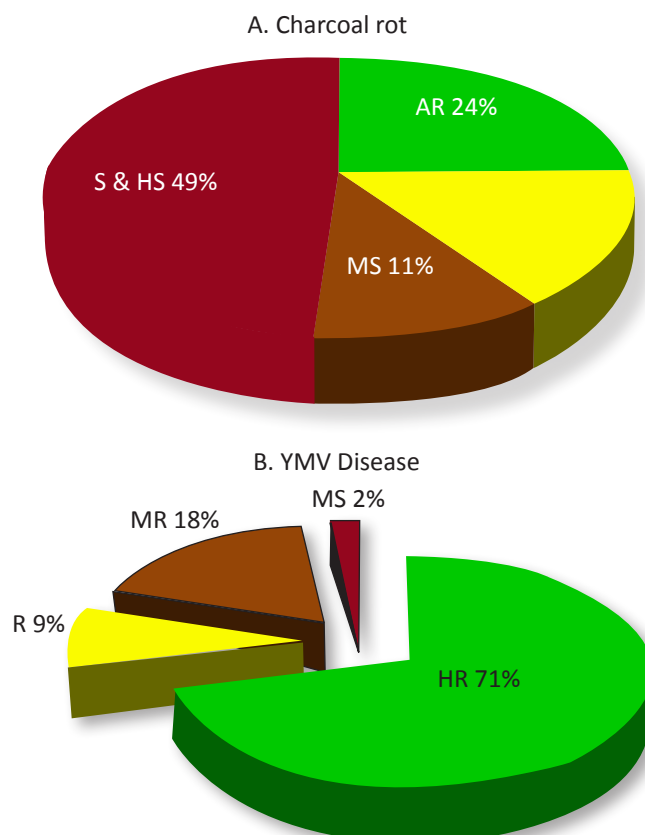


Figure 5: Distribution of genotypes on the basis of their reaction of resistance for Charcoal rot (A) and YMV Disease (B), respectively

mortality), moderately susceptible (10.1-25.0% mortality) and susceptible (25.1-50.0% mortality). Talukdar et al. (2009) also evaluated core set of 100 diverse genotypes for resistance against charcoal rot under paper towel methods and found that no genotypes were immune whereas only seven germplasm lines appeared to be resistant. Pancheshwari et al. (2016) recently screened different germplasm lines received from different part of country against YMV under Madhya Pradesh conditions and they found most of the genotypes reacted as highly resistant to whereas others exhibited moderately resistant to susceptible reactions to YMV. Das et al. (2017) also screened twenty six diverse genotypes and observed, only three varieties viz. PS 19, JS 97-52 and PK 564 as immune (Disease severity 0%) whereas JS 335 was found to be susceptible under West Bengal condition during *kharif*, 2016 and 2017. Recently, Amrate et al. (2018) also screened one hundred and nineteen genotypes, in this seventeen were multiple resistant against charcoal rot, YMV and aerial blight under Jabalpur, Madhya Pradesh condition.

## 4. Conclusion

The incidence of YMV was higher in early stage and started declining after 60 days of crop. Whereas charcoal rot was very

severe in later stage of crop and maximum plant mortality occurred in reproductive stages (60-75 days after sowing). Out of forty one promising entries, only six namely JS 21-17, AMS 100-39, RVS 2011-1, PS 1611, RSC 11-03 and SKF-SPS-11 were found to be absolutely/highly resistant to CR plus YMV. These promising genotypes can further be utilized for evaluation of resistance in other places as well as in resistance breeding programme.

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