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Studies On Pathogenicity Of *Macrophomina Phaseolina* On Sorghum Cultivars

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Abstract: *The test pathogen M.phaseolina was successfully isolated on Potato Dextrose Agar (PDA) medium. The pathogenicity of M.phaseolina was tested by sand culture method. Sand culture of the test pathogen was used as inoculum. After the inoculation the symptoms produced by test organism was observed both in roots and stems of sorghum cultivars employed at different plant growth stages. The present study reveals that the 100% disease incidence was observed at lodging and soft stalk stages, besides recording the maximum spread of pathogen up to 5nodes. Mean root infection also observed, based on this it has been established that CSV-8R is the susceptible cultivar and RS-29 is resistant cultivar to charcoal rot. The split open plants also revealed the presence of mycelia and conidia of fungus. This implies that M.phaseolina is the most important stalk rot pathogen.*

Key Words*: M .phaseolina , Susceptible , Resistant cultivar, Disease incidence ,Charcoal rot.*

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I. Introduction

Macrophomina phaseolina (Tassi) Goid. (= *Tiarosporella phaseolina* (Tassi) Van der Aa) is a soil borne plant pathogenic fungus. It belongs to the anamorphic Ascomycetes and is characterized by the production of both pycnidia and sclerotia in host tissues and culture media. The pycnidial state was initially named *Macrophoma phaseolina* by Tassi in 1901 and *Macrophoma phaseoli* by Maublanc in 1905. In 1927, Ashby maintained the name *Macrophomina phaseoli*, while Goidanich (1947) proposed *Macrophomina phaseolina. Tiarosporella phaseolina* (Tassi) Van der Aa was used in 1981 by Van der Aa to designate the species. Mihail (1992) indicated that there is an unconfirmed report of a teleomorph named *Orbilia obscura* (Ghosh et al., 1964) of *M. phaseolina*, but since then no further evidence appeared for the telemorph state. The sclerotial state was described for the first time by Halsted as *Rhizoctonia bataticola* (Taub.) Butler on *Ipomoea batatas* in 1890. According to Dhingra and Sinclair (1978), the same fungus was isolated from cowpea in India in 1912 by Shaw and was then named *Sclerotium bataticola*. Recently Crous et al. (2006) demonstrated that although the telemorph is unknown, *M. phaseolina* is a member of the family *Botryosphaeriaceae*. The authors pointed out the differences between *Tiarosporella* and *Macrophomina*, which produces in the pycnidia percurrently proliferating conidiogenous cells. The pycnidiospores are ellipsoid to obovoid, and measure (16-)20-24(-32) × (6-)7-9(-11) µm. During the sclerotial formation, 50–200 individual hyphal cells aggregate to give multicellular bodies named microsclerotia. The microsclerotia are black and are variable in size (50–150 µm) depending on the available nutrients of the substrate on which the propagules are produced (Short and Wyllie, 1978).

Macrophomina phaseolina incites a variety of diseases like seed rot, Seedling blight, Stalk rot and leaf spot in a wide variety of crop species belonging to dicots and monocots. Charcoal rot caused by this fungus has become common and it was found to be reduce the Sorghum ear head to about one-fifth of its normal size. Pathogenicity of the important fungal isolates was studied employing various inoculation techniques like Tooth pick method (Young, 1943) was widely used by various workers. Mesterhazy (1979) suggested stalk splitting as a method to evaluate stalk rot intensity in corn. Sick plot method and Tooth pick methods were evaluated by Anahousar (1983), who found no significant difference between the two. Anahousar and Patil (1983)and Shekar et al.,(1987) screened some newly released hybrids and showed that hybrids especially CSH 5 and CSH 6 were highly susceptible to stalk rot disease. Pande et al., (1989) also reported that there is no significant different in disease intensity either by Tooth pick method or by Sick plot method. Karunakar,(1989) Screened a set of 25 nonsenescences sorghum genotypes for varietal reaction against the infection of individual stalk rot fungi by Tooth pick method under field conditions. Several workers have attempted to screen sorghum lines including newly released hybrids to identify resistance.(Rao et al.,(1980); Zazzerini et al.,(1985); Anahousar and Patil,(1986); Pande et al .,(1989);Conn et al.,(1991);and Hiramath and Palakshappa (1994)). The Pathogenicity of various causal organisms against diseases of different economically important crop plants by several workers.

Fruit rot of chilli by Datar,(1995);Sclerotial wilt of castor bean by Grezes-Begget,(1996); Pythium rot of tomato by Bisht et al.,(1997); Charcoal rot of soybean by Smith and Carvil,(1997); Fusarium rot of cucumber by Ahn et al.,(1998); In pine spp by Banwart,(1998); In Sunflower by Suriachandraselvan and Seetharaman,(2003) and dry root rot of chick pea by Sayyad et al.,(2013).

II. Materials And Methods

The general laboratory techniques followed in this investigation were those as followed by Booth (1971)and Hawks worth(1974). A plot of 20x30 sq.ft was maintained at the fields of National Research Center for Sorghum (NRCS) , Rajendra nagar , Hyd.

Screening technique (Toothpick method) :

Eight (8) Sorghum cultivars were grown without irrigation in an environment known to be favorable for Charcoal rot, drought stress is induced by withholding irrigation at selected stages such as flowering to plant maturity and stalks are inoculated by inserting mycelium and sclerotia bearing toothpicks into holes made just above the first node. Amount of lodging, soft stalks and the spread of the fungus from the point of inoculation up the stem or the three measurements taken in assessing the reaction of genotypes to the Charcoal rot disease.

Isolation of the fungus:

The pathogen *M.phaseolina*(Tassi)Goid was isolated from the basal portion of the Charcoal rot effected Sorghum stem, collected from fields. Small bits of sclerotia bearing strands were surface sterilized by immersing in 0.1 percent mercuric chloride solution for two minutes. The surface sterilized strands were washed in three changes of sterile distilled water. They were planted on PDA under aseptic conditions and incubated at 25˚C in SEW , BOD incubator. After 4 days of incubation , the fungus was transferred on to a fresh PDA plate and was purified through single sclerotial isolation (Fig ; A&B)

Maintenance of the Fungus:

Purified culture of *M.phaseolina* was maintained on PDA slants at room temperature(26 – 28˚C).

Pathogenicity of *M.phaseolina* by Sand culture method:

Sand and culture was prepared by mixing sieved sand with Sorghum meal at 20:1 ratio and autoclaved at 15 p.s.i for one hour in two cycles after cooling the flasks were inoculated with test fungi and incubated. After 15days of incubation, this sand culture was mixed with field soil at 1:10 ratio and filled in alcohol sterilized plots. Surface sterilized sorghum seeds were sown in the soil. The pots were watered with sterile water on alternate days. Nutrients solution (Hoogland and Armon, 1950) was added on every 4^{th} day. Destructive sampling was done to see the presence of the fungus on the surface sterilized roots of the plant at periodical intervals on Czapek'sdox Agar medium. At the time of maturity the plants were split open to see the fungal damage and spread of the fungus in the stem (Fig: C)

Disease severity by employing Tooth picks method:

The most commonly used disease rating scale is 1 to 5 scale based on the percentage of lodged plants. (Where 1 - No Symptoms and 5- Severe infection), Frezzi and Tessandiar (1980).

III. Results And Discussion

Screening and Selection of Sorghum cultivar against Charcoal rot disease:

Four already released Sorghum genotypes(Swathi , CSV 8R , CSV 14R , M35-1) , one hybrid (CSH 13R) and its parental lines RS29 and 296 B and a resistant germplasm source E36-1 were screened by tooth pick method against charcoal rot disease in the fields at NRCS, Rajendra nagar Hyd. The above cultivars grown without irrigation in an environment known to be favorable for the spread of Charcoal rot disease. At selected stages of plant maturity and stalks are inoculated by inserting mycelium and sclerotia- bearing tooth picks into holes made just above the first node. Disease severity was calculated by using disease rating scale, (Frezzi and Tessandiar, 1980). Lodging, percentage of stalk rot and no. of nodes accrossed by the disease were considered as resistant parameters. Accordingly, the Sorghum genotypes were classified as highly susceptible (above 50% infection), susceptible (20 to 50% infection), moderately resistant (10 to 20% infection) and resistant (below 10% infection). The results are presented in a table. (Table-1)

Amount of lodging, soft stalks and the spread of the fungus from the point of inoculation up to the stem are the three measurements taken in assessing the reaction of genotypes to the charcoal rot disease. The data presented in table (Table -.2). Maximum infection was observed on CSV 8R, while RS29 had the lowest infection. Therefore, above 2cultivarsCSV 8R (susceptible) and RS29 (resistant) selected for further studies.

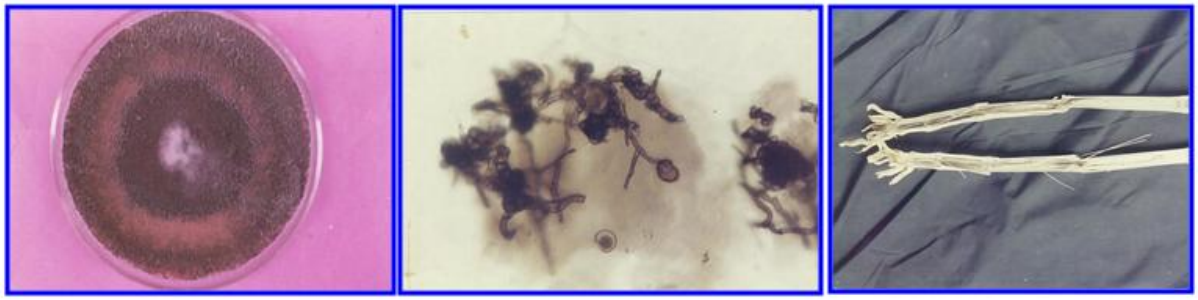
Pathogenicity of *M.phaseolina* on 2 sorghum cultivars (susceptible and resistant) by sand culture method:

Pathogenicity of *M.phaseolina* was tested on CSV 8R (susceptible cultivar) and also RS 29(resistant cultivar).sand culture of the test pathogen in viable state was used as inoculan.after the inoculation, the symptoms produced on test organism were observed both in roots and stems of the cultivar employed at different plant growth stages. The data presented in table (Table no 3).From the table; it is evident that the progress of Charcoal rot symptoms started from soft dough stage and reaching maximum symptoms production at physiological maturity stage. The symptoms produced on CSV8R, the susceptible cultivar are comparable to that of natural infected plants collected from field. However, the resistant cultivar has shown least infection both on root and stem. *M.phaseolina* being a stalk rot pathogen was able to show increased infection on stem in comparison with root.

Disease severity by sand culture method:

The data of disease severity is shown in table (Table no-4). Disease severity was measured by lodging, no of nodes crossed by the fungus and certain anatomical symptoms like pith disintegration, separation of vascular bundles and mycelial spread which are the standard criteria for evaluating the disease intensity of charcoal rot. From the table, it is clear that the 100% disease incidence was observed at lodging and soft stalk stages, besides recording the maximum spread of pathogen up to 5 nodes. Mean root infection also observed, based on this, it has been established that CSV8R is the susceptible cultivar to charcoal rot pathogen, while RS29 was comparatively resistant.

FIGURES



A B C

A) **Growth of *M. Phaseolima* – *In Vitro B) M. Phaseolima* (Tassi) Goid – 100 x 10 x**
C) Split open stalk of Susceptible cultivar – CSV 8R

TABLE- 1 Resistant parameter for Genotypes

Highly susceptible	Susceptible	Moderately resistant	Resistant
CSV 8R	Swathi	CSV 14R M35-1	RS29 E36-1 296-B CSH 13R

TABLE- 2 Screening of Sorghum Cultivars against Charcoal rot disease

Genotypes	Percent of lodging	Percent of Stalk rot	Mean no. of nodes crossed
E 36-1	8.45	13.00	0.261
CSV8R	100.00	98.15	5.00
M35-1	19.40	12.70	0.12
RS 29	6.40	10.30	0.01
CSV 14R	18.70	15.40	0.19
296B	10.50	12.58	0.25
CSH 13R	9.20	13.34	0.25
SWATHI	20.60	19.56	1.15

TABLE- 3 Pathogenicity test by sand culture method in the roots and stems of the both susceptible and resistant sorghum cultivars at different stages of plant growth

Plant growth stages	Percentage of infection			
	Susceptible (CSV 8R)		Resistant (RS 29)	
	Root	Stem	Root	Stem
Boot leaf	18	12	8	2
50% flowering	22	20	12	4
Soft dough	31	37	15	9
Hard dough	38	48	9	5
Physiological maturity	62	90	04	2

TABLE- 4 Disease severity of sorghum cultivar CSV8R Employing sand culture method

Lodging	100%
Soft stalk	100%
MNC	5
MRI	5
PD	4
VBS	4
FMS	4

MNC – Mean no. of nodes crossed, **MRI** – Mean root infection, **PD** – Pith Disintegration – 1-5 scale Where 1- No. symptoms, 2- 1-.25% (Low incidence), 3- 26-50% (Moderate incidence), 4- 51-75% (High incidence) 5- 76-100% (Severe incidence),**VBS** – Vascular bundle separation on 1-5 scale **FMS** – Fungal mycelium spread 1-5 scale Where 1- No symptoms 2- Initial symptoms 3- Slight disintegration 4-Moderate damage 5- Severe damage

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