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RESEARCH ARTICLE

Cultural, morphological, pathogenic and molecular diversity in *Macrophomina phaseolina* isolates of safflower from southern India

R.D. PRASAD*, T. NAVANEETHA and N. NAGESWARA RAO
Directorate of Oilseeds Research, Rajendranagar, Hyderabad 500 030

ABSTRACT: Isolates of *Macrophomina phaseolina* that causes root rot in safflower were collected from semi arid regions of Maharashtra (MS), Andhra Pradesh (AP) and Karnataka (K) states of India. Here we assessed the cultural, morphological, pathogenic and genetic affinity and diversity among twenty-four isolates of *M. phaseolina*. Principal component analysis (PCoA) of cultural and morphological parameters showed 13 groups which indicated high degree of variation among the isolates. The three major principal components considered for analysis explained 72% of the total variance. Among all the cultural characters studied variations were prominent in microsclerotia (shape, length and width) dimensions. Based on pathogenicity test of *M. phaseolina* isolates on susceptible safflower cultivars (cvs, 'A1' and NARI 6), the isolates were grouped into three aggressiveness groups (AG) *viz*., low, medium and highly aggressive. The *M. phaseolina* isolates prevalent in safflower growing areas showed variation in their virulence and that AG3 isolates can be utilized in screening safflower germplasm and breeding lines to identify resistance sources to *Macrophomina* root rot. Genetic diversity analysis using Random amplified polymorphic DNA (RAPD) markers and UPGMA cluster analysis distinguished the isolates into two major groups (Group I and Group II) and Group I has 23 isolates from safflower with a genetic similarity more than 86% among them and one isolate from castor formed a separate group with only 65% similarity Furthermore, the Group I was distinctly subdivided into two subgroups and one subgroup has all safflower isolates from Maharashtra region showing 88% genetic similarity. In general, a moderate genetic diversity was detected despite the complexity in genetic makeup among the isolates and this might be related to the predominant cultivation of safflower in these areas.

Key words: *Macrophomina phaseolina*, microsclerotia, pathogenicity, RAPD, safflower

The fungus *Macrophomina phaseolina* (Tassi) Goid a soilborne (Dhingra and Sinclair, 1978) and seed borne pathogen (Kunwar *et al*., 1986) distributed worldwide with a host range of more than 500 plant species (Mihail and Taylor, 1995). *Macrophomina phaseolina* infects roots and stems of a wide range of crops causing charcoal rot. Root rot caused by this pathogen has been considered as a relatively important disease in safflower (*Carthamus tinctorius* L.). A new stem splitting symptom in safflower caused by this pathogen has been reported recently and found that pathogen causes up to 25% mortality of plants in commercial fields of safflower (Govindappa *et al*., 2005). Planting resistant cultivars to manage *Macrophomina* root rot is economical and environmental friendly. However, developing safflower cultivars resistant to any particular disease requires source of resistance and information on variation in aggressiveness or virulence in pathogen population. Relatively little is known about the pathogenic variability of *M. phaseolina* isolates from safflower producing areas of India. An increased number of research workers have studied the pathogenicity of *M. phaseolina* by direct soil inoculation (Mayek-Perez *et al*., 2001) and or by root and crown inoculation at 3 weeks after germination (Prett *et al*., 1998). But pathogenicity study in pot culture experiments is laborious and time consuming. In our attempt of safflower root inoculation using infected tooth picks did not work in inducing root rot indicating non-fulfillment of Koch's postulates by *M. phaseolina* as a pathogen of mature safflower plants. (Srivastava *et al*., 2001) have reported a faster *in vivo* method based on germination towel technique for screening biocontrol agents against seed and root pathogens (Thiyagu *et al*., 2007) and have reported rapidness and ease of same technique in studying pathogenicity of *M. phaseolina* in sesamum. *M. phaseolina* is classified in the botryosphaeriaceae according to recent phylogenetic data which forms asexual structures like pycnidia and microsclerotia (Crous *et al*., 2006). The black, 0.1-1mm sized microsclerotia can survive upto 15 years depending on environmental conditions, and whether or not the sclerotia are associated with host residues (Cook *et al*., 1973; Papavizas, 1977; Short *et al*., 1980). Secondary dispersal by pycnidiospores is host and isolate-dependent (Ali and Dennis, 1992). *M. phaseolina* shows a great morphological (Mihail and Taylor, 1995) and genetic variability (Mayek-Perez *et al*., 2001; Su *et al*., 2001) which increases its adaptability to diverse environmental conditions. The first report of variability in morphological and virulence-based characters among isolates have been reported very long back (Dhingra and Sinclair, 1973) and techniques such as random amplified polymorphic DNA (RAPD) analysis have contributed to our understanding of genetic variability in populations of *M. phaseolina*. RAPD markers have been considered suitable for measuring genetic relatedness, detecting variation within and between *M. phaseolina* populations (Alvaro *et al*., 2003; Jana *et al*., 2003). It is also reported that isolates from a given host were genetically similar but differed from those of other hosts

*Corresponding author: ravulapalliprasad@gmail.com

(Su *et al*., 2001). Knowledge of field population of this pathogen from safflower is still scarce in India and till date only few detailed studies of field population has been published (Rajkumar and Kuruvinashetti, 2007; Jana *et al*., 2005). However, understanding of disease epidemiology and host-pathogen interactions is greatly dependent on knowledge of the diversity of the pathogen at the field level. The present work aims to understand the relationship among isolates of *M. phaseolina* based on cultural, morphological, pathogenic and genetic diversity and reaction of safflower cultivars to the this pathogen.

MATERIALS AND METHODS

Fungal isolates

Infected safflower plant samples were collected from root rot affected fields from Maharashtra, Andhra Pradesh and Karnataka states of India (Table 1).One isolate from castor crop (*Ricinus communuis* L.) was also obtained and the entire work has been carried out at Directorate of oil seeds Research, Hyderabad during the year 2008-09. Each infected root/stem portions were thoroughly washed and dried separately. *M. phaseolina* was isolated from different samples using standard isolation techniques on PDA. Plates were incubated at 26±1^{0}C in darkness for 6 days. Small individual colonies were collected along with small piece of medium and lightly ground in sterile water. A single microsclerotium was collected from each sample under microscope and transferred to PDA plates, separately. Pure culture of each isolate was obtained after 48 h of incubation, by selecting growing hyphae from a single sclerotium, grown on water agar plates, for microscopic confirmation of hyphal and conidial characteristics. Confirmed pure cultures were maintained on PDA slants at 26±1^{0}C for 6 days and stored in darkness at 4^{0}C for further use. A total of twenty-four isolates of *M. phaseolina* were isolated (twenty-three isolates from safflower and one from castor) and subjected to phenotypic and genotypic variability studies.

Table 1. Sources of *Macrophomina phaseolina* (Mp) isolates of safflower from India

Isolates	Districts	States	Variety/hybrid
K1	Dharwad	Karnataka	A1
AP2	Medak	Andhra Pradesh	Manjira
AP3	Rangareddy	Andhra Pradesh	Manjira
MS4	Hingoli	Maharashtra	PBNS 12
MS6	Hingoli	Maharashtra	PBNS 12
AP7	Hingoli	Maharashtra	Bhima
MS10	Parbhani	Maharashtra	NARI-NH-1
AP11	Parli	Maharashtra	PBNS 12
AP12	Parbhani	Maharashtra	PBNS 12
MS13	Parbhani	Maharashtra	PBNS 40
K14	Parbhani	Maharashtra	PBNS 40
MS15	Parbhani	Maharashtra	PBNS 40
K16	Parbhani	Maharashtra	PBNS 12
K17	Solapur	Maharashtra	Bhima
AP18	Akola	Maharashtra	Bhima
AP19	Solapur	Maharashtra	Bhima
K20	Raichur	Karnataka	A1
AP21	Rangareddy	Andhra Pradesh	Manjira
AP22	Rangareddy	Andhra Pradesh	NARI 6
K23	Raichur	Karnataka	A1
AP24	Medak	Andhra Pradesh	Manjira
AP25	Medak	Andhra Pradesh	Manjira
AP26	Medak	Andhra Pradesh	Manjira
AP27	Rangareddy	Andhra Pradesh	Manjira

Morphological diversity analysis

One mycelial disc (5mm diameter) from a seven days old culture was transferred to the PDA plate and incubated in the dark at 30^{0}C for 96h. Each treatment was replicated four times. The colony diameter and growth were recorded at 24h after incubation upto 96 h. On fifth day, main characteristics of the colonies were recorded (color of colony, presence/absence of aerial mycelium, shape and color of microsclerotia, colony shape). Finally, length and width of microsclerotia were recorded using a micrometer adapted to optical microscope (Carl Zeiss, Oberkochen, Germany) and shape and color of microsclerotia were recorded at 40X.

Analysis of aggressiveness in *M. phaseolina* isolates

Differences in aggressiveness among the twenty-four isolates of *M. phaseolina* were tested using germination towel technique based on root rot induced on susceptible safflower cultivars 'A1' and NARI-6. Seeds were surface disinfected with 2% sodium hypochlorite solution, followed by serial washings with sterile water. Surface disinfected seeds were inoculated with *M. phaseolina* isolates inoculum separately (approximately 2x10^3 cfu/seed). Untreated seeds served as check. For each treatment, 50 seeds were taken into account with 4 replications. The seeds were placed on the moist germination towels (germination towels were sterilized by dipping in mancozeb @ 0.2% solution) and incubated at 25^{0}C. On 7th day, the germination towels were opened without disturbing the roots, culture suspension of *M. phaseolina* was inoculated @2x10^{3}cfu/ml by smearing on to the roots using sterile cotton swab and incubated for 3 days at 25^{0}C. After incubation, root rot incidence was recorded and confirmed for microsclerotia formation in the infected roots. Finer root segments of 1 cm length was cut and placed on a glass slide over which a cover glass was placed and pressed gently and observed for microsclerotia under 40x using dark field microscope. Total number of microsclerotia produced was counted in 1cm length root fragment and all 5 roots were observed in each replication.

Genetic diversity analysis

Total genomic DNA extraction: Genomic DNA of *Macrophomina phaseolina* isolates was extracted by using the CTAB (Hexa- decy1 tri- methy1 ammonium bromide, Sigma Chemical CO., St. Louis, USA) method (Lee and Taylor, 1990). For DNA extraction, fungal mycelium were harvested from the isolates grown on potato dextrose broth

medium incubated for 3-5 days in orbital shaker (Kuhner, Switrzerland) at 200 rpm and 26°C. After incubation, the fungal biomass was filtered through sterile cheesecloth and was ground to a fine powder in the presence of liquid nitrogen. The extracted DNA was quantified by running 2µl of each DNA sample on 0.7% agarose gel with Lambda un cut DNA (30 and 60 ng) to get a final concentration of 15-20 ng/ml. To assess the quality, the DNA was diluted with TE buffer and loaded on 1% agarose gels in 0.5X TAE buffer (pH 8.0) in a gel electrophoresis apparatus (Biorad, USA) and electrophoresed for 30min under constant voltage (70 V). The ethidium bromide (final concentration of 0.3 mg/ ml) was added to the molten agarose at the time of gel casting. The gel was visualized on a UV transilluminator, and photographs were taken using a Gel Documentation System (Biorad, USA).

RAPD analysis

DNA samples of all the twenty-four isolates of *Macrophomina phaseolina* were subjected to PCR-RAPD analysis by amplifying the genomic DNA using four random primers OPA 2 (5'- TGCCGAGCTG-3'), OPA 9 (5'-GGGTAACGCC-3'), OPC 1 (5'TTCGAGCCAG-3') and OPC 10 (5'-TGTCTGGGTG-3') which were selected after initial screening of 60 primers of operon A, B and C series (Operon Technologies Inc., Alameda, CA, USA). The amplification reactions with OPA primers were carried out in a final volume of 15 µl of reaction mixture containing 5 ng of genomic DNA and 1.5µl of a 10 X PCR buffer, 0.6µlof dNTP's, 0.8µl of primers and 0.15µl of *Taq* polymerase (Bangalore Genei). The PCR amplifications were performed using Thermal Cycler (Biorad) programmed for initial denaturation at 94°C for 3min followed by 45 cycles at 92°C for 30sec, 36°C for 45sec, 72 °C for 2 min and a final cycle at 72°C for 10 min. All amplified DNA products were resolved by electrophoresis on agarose gel (1.5%) in TAE (1X) buffer, stained with ethidium bromide and photographs were taken using Gel Documentation System.

Data analysis

Mean ± standard error (SE±) was calculated for morphological (Length, width and length / width of microsclerotia) data of *M. phaseolina* isolates. All morphological data were subjected to Principal Component Analysis (PCoA) based on 3 principal components calculated and two dimensional PCoA plot was obtained. The PCR-RAPD amplification pattern was converted into binary data matrix, the presence of band at an amplicon level was scored as 1 and its absence as 0. The binary data was analyzed using standard procedure in NTSYS-PC (version 2.1; Exeter Biological Software, Setauket, NY) software package (Rholf, 2000). Clustering analysis was performed with the Unweighted Pair-Group Method using Arithmetic averages (UPGMA) in the SAHN module of NTSYS-PC.

RESULTS AND DISCUSSION

In the present study, the results obtained showed considerable variation among morphological, pathogenic and genetic variability of the isolates. Even though only one species was recognized within the genus *Macrophomina* (Mihail, 1992), great variability in cultural morphology, the pathogenicity and on the molecular level were recorded on which efforts were made worldwide to characterize and classify *Macrophomina phaseolina* isolates (Atiq *et al.,* 2001; Almeida *et al.,* 2003; Beas-Fernandez *et al.,* 2006).

The shape of fungal colony varied from round, ovoid to irregular; average to high mycelium production in most of the isolates. Color of the mycelium was grey in all isolates except in the isolate, *M. phaseolina* AP3, which was black color of microsclerotia ranged from brown to black (Table 2 and 3). However, no significant differences were found among the isolates in morphological characters like length, width and length/width ratios of microsclerotia except the isolate, AP 21 that did vary in length/width ratio. PCoA of two qualitative (shape, aerial mycelium) and seven quantitative (colony diameter, length, width and length/width of microsclerotia) characteristics formed 13 groups (Table 4). The three major principal components explained 72% of the total variance. These results suggested the prevalence of high degree of variability in the morphological variables subjected for analysis in *M. phaseolina* isolates. The pathogenic variability studied using germination towel technique clearly showed differences in the level of virulence of *M. phaseolina* isolates tested (Table 5).

The genetic divergence recorded among the 24 isolates of *M. phaseolina* through PCR-RAPD analysis was informative. Dendrogram generated by cluster analysis presented the isolates with varied levels of genetic similarity ranging from 65-100% (Fig. 1 and Fig. 2). The UPGMA cluster analysis exhibited two major groups designated as Group I and Group II and a single isolate forming as an outgroup. The isolates in Group I and Group II shared the percentage of similarity ranging from 89 to 98% and 87 to 100% respectively and the out group (Ap 24) just shared 64% similarity among the other isolates. Overall, moderate genetic diversity was apparent despite high complexity in genetic variation among the isolates which can be perceived as distinct subdivision of Group I into two subgroups as subgroup 1 and subgroup 2. Again the subgroup 1 was distinctly subdivided into 3 subclades designated as a, b and c in the dendrogram. Likewise, Group 2 was also distinctly subdivided into two subgroups as 1 and 2. This suggests that RAPD primers employed has high discriminatory power probably by picking polymorphism from the most variable regions of the genome. This result indicates the existence of distinct genetic background among the twenty four isolates of *M. phaseolina*. Thus it indicates that RAPD markers have again proved to be suitable for measuring the genetic similarity and diversity among *M. phaseolina* isolates. Similar kind of genetic differences among the isolates of *M. phaseolina* was observed by (Jana *et. al.,* 2003) them and they concluded that it is due to their isolation from diverse hosts and distinct geographical regions. Although, the isolates of *M. phaseolina,* used in their study, showed morphological similarities (Jana, 1998), they exhibited a wide range of genetic variability with RAPD markers. With a few isolates

Table 2. Variation in aerial mycelium formation and colony diameter among 24 isolates of *M. phaseolina*

Isolates no.	Colony characteristcs		Colony diameter (mm)			
	Color	Aerial mycelium	24h	48h	72h	96h
K1	Grey	++	10.3	21.0	24.6	31.3
AP2	Grey	++	10.0	21.3	25.0	31.6
AP3	Black	++	7.6	19.6	26.6	30.0
MS4	Grey	++	7.6	19.6	26.6	30.0
MS6	Grey	+++	12.6	31.0	29.0	42.6
AP7	Grey	++	5.0	16.6	25.3	32.0
MS10	Grey	+	1.0	6.6.0	21.3	29.6
AP11	Grey	++	9.6	21.6	28.0	32.3
AP12	Grey	+	11.3	23.3	27.3	37.0
MS13	Grey	++	6.6	19.0	25.0	31.0
K14	Grey	++	10.6	20.0	26.0	30.0
MS15	Grey	+++	12.6	31.0	29.0	42.6
K16	Grey	+	6.3	17.0	20.3	26.3
K17	Grey	++	10.6	24.6	28.3	38.6
AP18	Grey	+++	14.0	29.6	28.6	39.3
AP19	Grey	++	8.3	21.0	25.3	32.3
K20	Grey	+	6.3	20.0	23.4	30.3
AP21	Grey	+	1.6	13.6	20.3	29.0
AP22	Grey	++	10.3	21.6	27.0	35.0
K23	Grey	++	10.0	22.3	26.0	33.6
AP24	Grey	++	9.3	22.3	28.3	35.0
AP25	Grey	++	14.3	28.6	31.3	40.3
AP26	Grey	+	8.3	22.0	24.1	31.3
AP27	Grey	+	7.0	18.0	25.3	33.6

Indices: + = poor; ++ = average; +++ = high; K = Karnataka, AP = Andhra Pradesh

Table 3. Variation in abundance, growth, color, shape and size of microsclerotia among 24 isolates of *M. phaseolina*

Isolates	Abundance	Color	Shape	Length (µm)	Width (µm)	Length/ Width
K1	++	Brown	Oviod	155 ± 18.0	146.7 ± 15.3	1.0 ± 0.1
AP2	++	Brown	Round	158 ± 14.4	130.0 ± 32.4	1.2 ± 0.4
AP3	++	Brown	Round	155.0 ± 8.7	155.0 ± 8.7	1.0 ± 0.0
MS4	++	Black	Oviod	250.0 ± 43.0	143.3 ± 11.5	1.7 ± 0.2
MS6	++	Brown	Oviod	133.3 ± 28.9	150.0 ± 10.0	0.8 ± 0.1
AP7	++	Brown	Round	113 ± 5.0	113.3 ± 12.6	1.0 ± 0.1
MS10	+++	Black	Oviod	108.3 ± 14.4	120.0 ± 26.5	0.9 ± 0.2
AP11	+	Brown	Irregular	196.7 ± 5.8	210.0 ± 26.5	0.9 ± 0.1
AP12	+	Brown	Irregular	201.7 ± 162.6	305.0 ± 39.7	0.7 ± 0.6
MS13	++	Brown	Oviod	341.7 ± 38.2	321.7 ± 30.1	1.0 ± 0.1
K14	++	Brown	Round	138.3 ± 12.6	163.3 ± 32.1	0.8 ± 0.1
MS15	++	Brown	Oviod	133.3 ± 28.9	150.0 ± 10.0	0.8 ± 0.1
K16	++	Brown	Irregular	145.0 ± 15.0	135.0 ± 15.0	1.0 ± 0.1
K17	++	Brown	Irregular	330.0 ± 60.8	346.7 ± 95.7	1.0 ± 0.2
AP18	+++	Brown	Oviod	300.0 ± 66.1	283.3 ± 57.7	1.0 ± 0.0
AP19	+++	Brown	Irregular	346.7 ± 105.0	358.3 ± 76.4	0.9 ± 0.0
K20	+++	Brown	Oviod	245.0 ± 18.0	181.7 ± 2.9	1.3 ± 0.1
AP21	++	Black	Irregular	483.3 ± 28.9	200.0 ± 0.0	2.4 ± 0.1
AP22	++	Brown	Oviod	211.7 ± 12.6	216.7 ± 62.9	1.0 ± 0.3
K23	++	Brown	Round	105.0 ± 8.7	108.3 ± 14.4	0.9 ± 0.0
AP24	+++	Black	Oviod	258.3 ± 38.0	205.0 ± 48.2	1.2 ± 0.2
AP25	+	Brown	Irregular	200.0 ± 50.0	221.7 ± 64.5	0.9 ± 0.0
AP26	+	Brown	Irregular	168.3 ± 28.4	113.3 ± 23.1	1.5 ± 0.4
AP27	+++	Black	Irregular	338.3 ± 12.6	243.3 ± 20.2	1.4 ± 0.1

Index: + = poor; ++ = average; +++ = high; K = Karnataka, AP = Andhra Pradesh, MS= Maharashtra ; Mean± Standard error.

Table 4. Eigen values associated to the 3 principal components from Principal Component Analysis (PCoA) based on *in vitro* characteristics of 24 isolates *Macrophomina phaseolina*

Characteristics	Principal components		
	1	2	3
Length	-.150945	0.617670	0.375823
Width	0.072511	0.626264	-.039924
LW	-.294788	0.162578	0.510903
hr24	0.434723	0.061379	0.049405
hr48	0.425750	0.110234	0.047141
hr72	0.435813	0.092143	0.001621
hr96	0.411537	0.117062	0.011800
Aerial	0.384284	-.081520	0.382550
Shape	-.088548	0.393811	-.667077

Su *et. al.,* (2001) have shown genetic relatedness of *M.phaseolina* according to their host specialization. Beas *et al.* (2006) though demonstrated low frequency of anastomosis among the isolates of *M. phaseolina*, they found high morphological and pathogenic variability and presumed that morphological and pathogenic differentiation among the isolates could be produced for some reproductive isolates based on geographical origins. The predominant cultivation of safflower in these regions could also be attributed to the existence of wide morphological, pathogenic and moderate genetic diversity among the isolates because of low frequency of hyphal fusion. In general, positive correlation between the isolates in the groups and their geographical origin was observed to a certain extent. This positive correlation between the isolates in genetic diversity groups and their geographic origin obtained from Maharashtra where safflower being grown more predominantly might be concluded as the dissemination of the organism through seed exchange in that region as this fungus is seed transmitted. The pathogen also spreads through contaminated equipments and soil with sclerotia. Such high similarity was also observed among Brazilian isolates from different regions (Alvaro *et al.*, 2003) and they presumed that the isolates used in the work did not evolve independently from each other and considered as part of the same ancestral population. They also did survey at closely located areas and had emphasized the importance of intensive survey. Therefore, in our case also intensive sampling has been done to determine the gene flow among population of this fungus, in semiarid regions. No positive correlation was observed between the isolates in the groups that were clustered based on the PCoA of morphological variables and genetic relation which supports the claim of Su *et al.* (2001), that isolates with similar morphology need not be genetically identical. The isolate, Ap 24 presented high genetic divergence which might indicate the specificity of the isolate to castor cultivars probably due to monocroping of castor in A.P. state.

Table 5. Twenty four *M. phaseolina* isolates, their different level of aggressiveness groups (AG) based on % root rot incidence on safflower cultivars

Aggressiveness groups (AG)	cv. A 1	cv. NARI 6
AG 1 (< 25%)	K 1, AP 2, AP 3, MS 10, AP 11, AP 12, K 14, MS 15, MS 16, MS 18, MS 19, K 20, AP 21, AP 22, AP 25, AP 26.	K 1, AP 2, AP 3, MS 6, MS 10, AP 11, AP 12, K 14, MS 15, MS 16, MS 18, K 20, AP 21, AP 22, K 23, AP 25, AP 26, AP 27
AG 2 (25-50%)	MS 6, AP 7, MS 13, MS 17, K 23, AP 24, AP 27,	AP 7, MS 17, MS 19, AP 24, MS 13
AG 3 (> 50%)	MS 4	MS 4

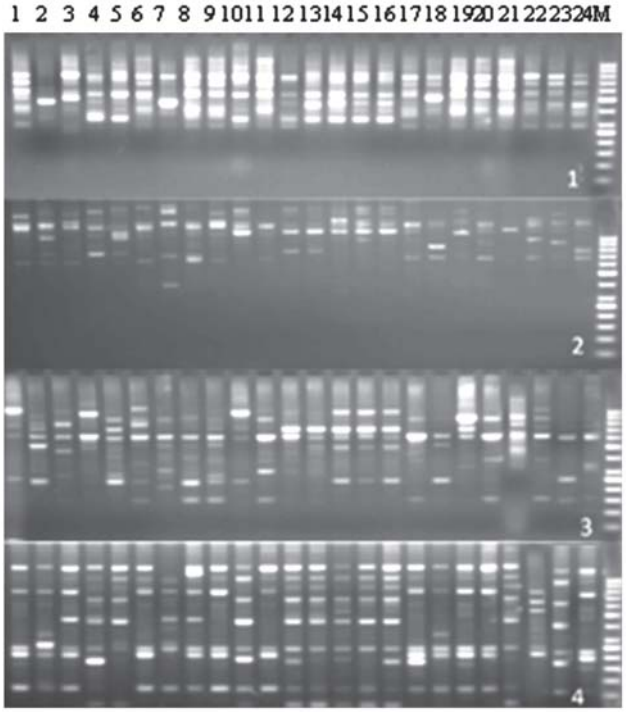

Fig. 1. Banding pattern of Random amplified polymorphic DNA (RAPD) obtained from 24 isolates of *M. phaseolina*. Lane 1=k1, 2= AP 2, 3= AP 3, 4= MS4, 5= MS6, 6= AP7, 7= MS10, 8= AP11, 9= AP12, 10= MS13, 11= K14,12= MS15, 13= K16, 14=K17, 15=AP18, 16= AP19, 17=K20, 18= AP21, 19=AP22, 20=K23, 21=AP24, 22=AP25, 23=AP26, 24=AP27. M=100 bp ladder with primers OPA-2(1), OPA-9(2), OPC-1(3) and OPC-10(4)

The isolates differed significantly for their aggressiveness and the 24 isolates were categorized into low aggressive (AG 1), medium aggressive (AG 2), and highly aggressive (AG 3) groups based on % root rot induced on susceptible cultivars A1 and NARI 6 of safflower (Table 5). Majority (58%) of the isolates were grouped in AG 1 and six isolates were grouped in AG 2. Only three isolates (MS 4, MS 13 and K 23) were found to be in AG 3 as they induced root rot more than 50%. Minor variation in degree of

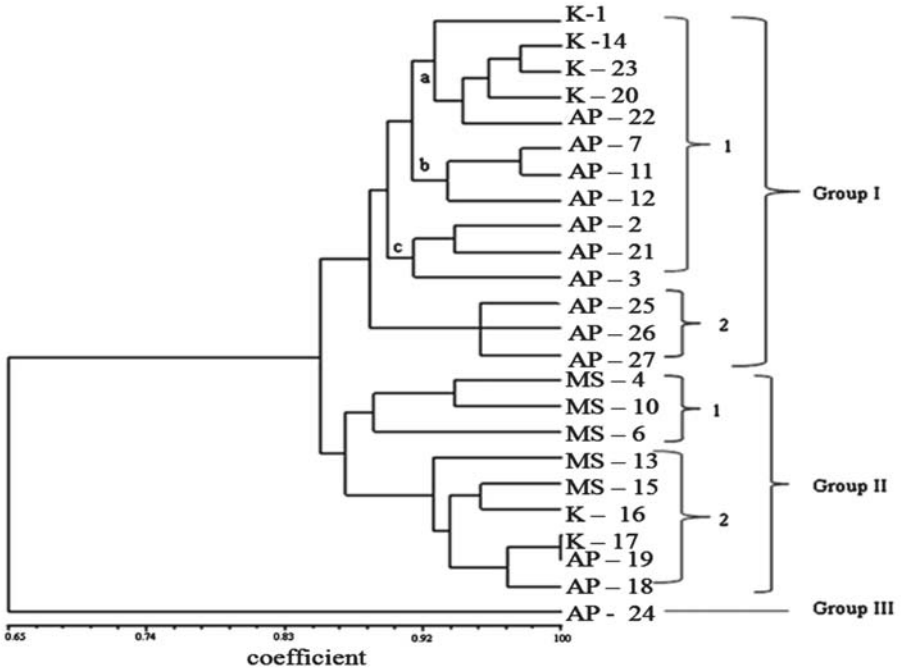

Fig. 2. Dendrogram constructed with UPGMA clustering method among 24 isolates of *M. phaseolina* isolates based on polymorphism obtained with RAPD primers. The branches are labeled by isolates and their geographical origin.

aggressiveness was observed on NARI- 6 with MS 6, MS 19, MS 10 and AP27 and rest of the isolates showed similar pathogenic reaction as in with cv. A-1. Most of the isolates collected from Karnataka were found to be less aggressive. No relationship was observed between the isolates in the aggressiveness groups, the morphological and genetic diversity groups.

In conclusion, our study demonstrated the obvious difference in morphology, pathogenicity and genetic makeup among *M. phaseolina* isolates of South India. This diversity may be related to the predominant cultivation of safflower and different varieties of the crop in these regions. The success of pathogenicity test indicates the applicability of germination towel technique as a reliable and rapid method to determine pathogenicity among isolates of *M. phaseolina* in safflower and to screen the safflower genotypes for their susceptibility or resistance to *M. phaseolina.* Distinct pathogenic variability among indigenous *M. phaseolina* isolates was detected and aggressive group AG3 isolates (MS 4, MS 13 and K 23) can be utilized in screening of safflower germplasm and breeding lines for resistance to Macrophomina root rot.

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REFERENCES

Ali, S.M. and **Dennis, J.** (1992). Host range and physiologic specialization of *Macrophomina Phaseolina* isolated from field peas in South Australia. *Aust. J. Exp. Agric*. **32**: 1121-1125.

Alvaro, M.R.A., Ricardo, V.A., Carlos, A.A.A., Valdemar, P.C., David, S.J.F., Silvana, R.R.M., Luis, C.B., Mauro, C.P. and **Claudio, G.P.C.** (2003). Genotypic diversity among isolates of *Macrophomina phaseolina* revealed by RAPD. *Fitopatol. Bras.* **28**: 279-285.

Atiq, M., Shabeer, A. and **Ahmed, I.** (2001). Pathogenic and cultural variation in *Macrophomina phaeolina,* the cause of charcoal rot in sunflower. *Sarhad J. Agric*. **2**: 253-255.

Beas- Fernandez R., ADe Santiago–De Santoiago, Hernandez–Delgado S. and **Mayek–Perez, N.** (2006). Characterization of Mexican and Non-Mexican isolates of *Macrophomina phaseolina* based on Morphological characterizatics, Pathogenicity on bean seeds and endoglucanase genes. *J. Plant Pathol.* **88(1)**: 53-60.

Cook, G.E., Boosalis, M.G., Dunkle, L.D. and **Odvody, G.N.** (1973). Survival of *Macrophomina phaseolina* corn and sorghum stalk residue. *Plant Dis. Rep*. **57**: 873-875.

Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marasas, W.F.O., Philips, A.J.L., Alves, A., Burges, T., Barber, P. and **Groenewald, J.** (2006). Phylogenetic lineages in the Botryosphaiaceae. *Studi. Mycol*. **55**: 235-253.

Dhingra, O.D. and **Sinclair, J.B.** (1973). Variation among isolates of *Macrophomina phaseolina* from different region. *Phytopathology* **76**: 200-204.

Dhingra, O.D. and **Sinclair, J.B.** (1978). Biology and Pathology of *Macrophomina phaseolina*. Universidade Federal De Vicosa, Vicosa Brazil. pp. 141-166.

Govindappa, M., Lokesh, S. and **Rai, V.R.** (2005). A new stem splitting symptom in safflower caused by *Macrophomina phaseolina*. *J. Phytopathol*. **153**: 560-561.

Jana, T.K. (1998). Agglutination potential of *Pseudomonas fluorescens* to *Macrophomina phaseolina* and its ecological significance. Ph.D. Thesis, Banaras Hindu University, India.

Jana, T., Sharma, T.R., Prasad, R.D. and **Arora, D.K.** (2003) Molecular characterization of *Macrophomina phaseolina* and *Fusarium* species by a single primer RAPD technique. *Microbiol. Res*. **158**: 249-257.

Jana, T., Singh, N.K., Koundal, K. and **Sharma, T.R.** (2005). Genetic differentiation of charcoal rot pathogen, *Macrophomina phaseolina*, into specific groups using URP-PCR. *Can. J. Microbiol.* **51**: 159-164.

Kunwar, I.K., Singh, T., Machado, C.C. and **Sinclair, J.B.** (1986). Histopathology of soybean seed and seedling infection by *Macrophomina phaseolina*. *Phytopathology* **76**: 532-535.

Lee, S.B. and **Taylor, J.W.** (1990). Isolation of DNA from fungal mycelia and single spores. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR protocols: A guide to methods and applications, Academic press, SanDiego. pp 282-287.

Mayek-Perez, N., Lopez-Castaneda, C., Gonzalez-Chavira, M., Garcia–Espinosa. R., Acosta–Gallegos, J.A., Matinez-De La Vega O. and **Simpson, J.** (2001). Variability of Mexican isolates of *Macrophomina phaseolina* on the basis of pathogenesis and AFLP genotype. *Physiol. Mol. Plant Pathol.* **59**: 257-264.

Mihail, J.D. (1992). *Macrophomina*. *Methods for Research on Soil-borne Phytopathogenic Fungi,* Singleton, L., J. Mihail and C. Rush (Eds.). American Phytopathology Society, St. Paul, USA.

Mihail, J.D. and **Taylor, S.J.** (1995). Interpreting of variability among isolates of *Macrophomina phaseolina* in pathogenicity, pycnidium production and chlorate utilization. *Can. J. Bot.* **73**: 1596-1603.

Papavizas, G.C. (1977). Some factors affecting survival of sclerotia of *Macrophomina phaseolina* in soil. *Soil Biol. Biochem*. **9**: 337-341.

Prett, R.G., McLaughlin, M.R., Pederson, G.A. and **Rowe, D.E.** (1998). Pathogenicity of *Macrophomina phaseolina* to mature plant tissues of Alfalfa and white clover. *Plant Dis*. **82**: 1033-1038.

Rajkumar, Fakrudin, B. and **Kuruvinashetti, M.S.** (2007). Genetic variability of sorghum charcoal rot pathogen (*Macrophomina phaseolina)* assessed by random DNA markers. *Plant Pathol. J.* **23**(2): 45-50.

Rholf, F.J. (2000). NTSYS-pc Numerical taxonomy and multivariate system, version 2.1. Exeter publishing, Setauket, USA.

Short, G.E., Wyllie, T.D. and **Bristow, P.** (1980). Survival of *Macrophomina phaseolina* in soil and residue of soybean. *Phytopathology* **70**: 13-17.

Su, G., Suh, S.O., Schneider, R.W. and **Russian, J.S.** (2001). Host specialization in charcoal rot fungus, *Macrophomina phaseolina*. *Phytopathology* **91**: 120-126.

Srivastava, R.K., Prasad, R.D., Rangeshwaran, R., Wasnikar, A.R., Singh, S.P. and **Rao, N.S.** (2001). A rapid *in vivo* bioassay method for testing and selection of fungal antagonists of plant pathogens. *J. Biol. Cont.* **16**: 173-176.

Thiyagu, K., Kandasamy, G., Manivannan, N., Muralidharan, V. and **Manoranjitham, S.K.** (2007). Identification of resistant genotypes to root rot disease (*Macrophomina phaseolina*) of sesame (*Sesamum indicum* L.). *Agricultural Science Digest* Year: 2007.

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