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Genetic variability among South Indian isolates of *Macrophomina phaseolina,* the fungus involved in charcoal rot of mulberry and association of pathogenicity

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Abstract
This study reports the results of a survey conducted in an important sericulture area in South India, collecting a fungus associated with charcoal root rot- *M. phaseolina.* A total of 35 strains were recovered from mulberry gardens from Tamil Nadu, Andhra Pradesh, Karnataka and Telangana, showing varying degrees of disease severity. Isolates showed considerable variability in cultural, morphological and pathogenic traits but high RAPD and SSR Marker polymorphisms were recorded. PCR amplification of 44 RAPD primers generated 501 markers showed nearly similar genetic distance measures. Clustering based on both phenotypic and genotypic markers failed to correlate either the geographical distribution or pathogenicity of the isolates. Associations between markers and isolate virulence were established by stepwise multiple regression analysis, providing insight into genomic regions that contribute to fungal virulence. We conclude that the genetic diversity and diversity of fungi is high and therefore systematic approaches are needed for breeding for disease resistance in host plants.

Keywords: *M. phaseolina*, mulberry, charcoal root rot, pathogenicity, genetic variability, RAPD, SSR

Introduction
One of the most significant soil-borne pathogens is the fungus *Macrophomina phaseolina* (Tassi) Goid, which produces pycnidia and infects more than 500 plant species from more than 100 plant families globally (Ndiaye *et al*. 2010) [25]. It causes charcoal rot in mulberries (*Morus* spp.), a serious disease that is favoured by hot, dry weather or when plants are stressed by unfavourable environmental circumstances (Chowdhary 2006) [41]. The preferred method for determining whether a plant is infected with charcoal rot caused by *M. phaseolina* is visual examination of the exterior symptoms on the plant's roots and overall plant. Charcoal rot develops during cultivation in mature gardens as well as in nurseries, resulting in crop loss and financial harm. Due to their epidemic nature and potential to destroy the plant, other types of root rot diseases, such as dry root rot (*Fusarium solani* (Mart.) Sacc. and *F. oxysporum* Schlecht.), black root rot (*Botryodiplodia theobromae* Pat.), are also observed (Sharma *et al.* 2003) [24]. According to Chowdhary (2006) [41], Karnataka (12.3 - 39.3%), Tamil Nadu (14 - 71%), and Andhra Pradesh (22.6-73.3%) were the states with the greatest disease incidence rates. Millions of metric tonnes of soybean yield were estimated to have been lost in the United States as a result of charcoal rot (Wrather and Koenning 2006) [39], but losses from charcoal rot were reported to range from 60 to 90% in other crops, such as sunflower (Khan 2007) [18]. The symptoms of charcoal rot in adult plants are followed by wilting due to the closure of xylem arteries, which results in death, making chemical management of the disease challenging. Microsclerotia and pycnidia, two asexual structures, are produced by *M. phaseolina*. Pycnidia produced in infected host tissue that were later released into the soil during the decaying process allow the fungus to survive and serve as a conidial inoculum for secondary dissemination (Khan 2007) [18]. M. *phaseolina* causes cortical and vascular discolouration in a variety of hosts by infecting the root, basal stem, and collar area. However, pathogen diversity has an impact on disease epidemiology and host-pathogen interactions. Both morphological studies and molecular techniques have been used to characterize isolates of *M. phaseolina* from various legumes (Babu *et al.,* 2007; Sharma *et al*., 2003) [42, 24]. Identification of *M. phaseolina* based on cultural and morphological features such as colony morphology, micro sclerosis, conidia and conidia microscopy is poor (Saleh *et al*., 2010) [43].

Chemical control of the fungus is impractical due to its high level of diversity, soil-borne habit, and capacity for survival. High levels of diversity are common in fungal populations, which enhances their capacity to adapt to various circumstances and get beyond host resistance (Trigiano *et al.* 2008) [35]. In order to generate resistant mulberry cultivars using the host gene pool, a plant breeding programme and genetic diversity assessment of the pathogen are necessary. To describe the genetic and pathogenic variability of *M. phaseolina*, several investigations have been conducted. The genetic and pathogenic variability among populations of this fungus has been better understood thanks to advanced molecular techniques like Restriction Fragment Length Polymorphism (RFLP) and PCR-based technology like Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP). Almeida *et al*. (2003) [1] came to the conclusion that RAPD markers were helpful in determining genetic relatedness and spotting variation within and across populations of *M. phaseolina*, aiding in the comprehension of the ecology and biology of the pathogen. In order to connect 45 isolates of *M. phaseolina* from soybean, cotton, sorghum, and corn, Su *et al.* (2001) used RAPD markers. They were able to unambiguously group isolates from different sources into different clusters. Therefore, the use of molecular markers in the biological functions of *M. phaseolina* can provide insights for understanding host-pathogen relationships. This study was conducted with the following main objectives: (ii) characterization of isolates by cultural, morphological and molecular markers (RAPD and SSR) and (iii) assessment of the association of virulence and its markers.

Materials and Methods

Survey and collection

Different mulberry growing areas of Karnataka, Andhra Pradesh, Tamil Nadu and Telangana were surveyed and root rot disease incidences were recorded. Both infected and healthy root samples were collected from different locations. Root bits of the samples were surface sterilized and placed on potato dextrose agar (PDA) medium. After 3 days of inoculation, the *M. phaseolina* isolates were identified based on morphological characteristics. Table 1 shows the list of *M. phaseolina* isolates recovered after culturing. The isolates were purified by mono-hyphal tip method (Rangaswami and Mahadevan 2008) [29]. These isolates were stored on PDA at 4 ºC for further use in characterization and pathogenicity testing.

Cultural and morphological characterization

One mycelial block (8 mm diameter) from a seven-day-old pure culture was transferred to fresh Petri plate containing PDA. Mean diameter of the colony (in mm) was recorded in every 24 hours interval and relative growth rate of colony was calculated (mm/day) (Mayek-Perez *et al.* 1997) [21]. On the fifth day, characteristics of the colony were recorded (colour of the colony, aerial mycelial growth, colour of the mycelium, shape of pycnidia and their abundance).The isolates were characterized using morphological and cultural parameters (Butler 1981) [10]. Mass multiplication of fungal isolates and pathogenicity testing the axenic culture of each fungal isolates was mass multiplied on suitable media/substrate (i.e., boiled sorghum) and inoculated on V-1 mulberry plants grown in pots in triplicates and uninoculated plants served as control. All pots were shifted to the green house and arranged in Complete Randomized Design (CRD). Observations on root rot symptoms *viz*., wilting and survival of the plants from 15 days onwards till 90 days were recorded. The root rot indices were calculated by sacrificing the plant on 90th day of inoculation. The pathogen was re-isolated from the infected roots to confirm its pathogenicity and to prove the Koch's postulates (Koch 1890) [19]. Categorization of pathogens into virulent, moderately virulent, and avirulent was based on root rot infection (%) and leaf witling (%) after 90 days of inoculation.

Genomic DNA isolation

Genomic DNA was isolated from pure culture of each isolate using mycelial mat harvested by filtration through Whatman filter paper. The mycelial mat was thoroughly and repeatedly washed with distilled water and dried using tissue paper. Mycelial mat (~150 mg) was ground in liquid nitrogen with the help of mortar and pestle. DNA was isolated from using Hipura TM Fungal Genomic DNA Extraction Kit (Hi-MEDIA, Mumbai, India) by following manufacturer's instructions. The genomic DNA was quantified on 0.8% agarose gel stained with Ethidium Bromide (EtBr) and uniformly diluted to 10 ng/µl for PCR amplification.

RAPD marker amplification

RAPD-PCR was carried out using the protocol of Williams *et al*. (1990) with minor modification. A preliminary screening of RAPD primers (Operon Technologies, USA) was carried out using a subset of 6 fungal isolates to select the polymorphic ones. A total of 44 polymorphic primers were used for profiling *M. phaseolina* isolates. The PCR reactions carried out in a 20 µl reaction mixture containing 1 X PCR buffer with 2.0 mM MgCl2, 0.1mM dNTPs, 0.5 U of Genie Taq DNA Polymerase (Merck, India), 0.2 µM/µl primer and 20 ng genomic DNA of the fungal isolate. PCR amplification involved an initial denaturing step of 94 ºC for 3 min followed by amplification programmed for 40 cycles in PCR Thermocycler (Applied Biosystems, USA). Each cycle consisted of a denaturation step at 94 ºC for 1min, a primer annealing at 35 ºC for 1min, a primer extension step for 2 min and a final polishing step of 72 ºC for 7 min. The amplified DNA fragments were separated on 1.5% agarose gel in 1 X TAE (Sambrook and Russel 2001) [30], and documented using Gene Genius gel documentation system (Syngene, UK). λ DNA/EcoRI+HindIII marker (Fermentas, Lithuania) was used as a size standard.

SSR marker amplification

A total of 57 *M. phaseolina* and *Fusarium* specific microsatellite or simple sequence repeat (SSR) primers were synthesized based on the information available in the literature and which are already submitted to the GenBank, NCBI (Arias 2011; Bogale *et al*. 2005; Baird *et al*. 2009; Mwang'ombe *et al*. 2008; Bahar and Shahab 2012; Datta and Lal 2013) [4, 9, 7, 23, 6, 14]. All synthesized primers were screened and optimization for PCR amplifications was carried out using a subset of 6 fungal isolates. Based on clear amplifications, 25 primers were identified for genetic profiling. The PCR amplifications of were carried out in 10 µl reaction volume, containing 10 ng template DNA, optimized concentration of each primer, 2 mM of MgCl2, 0.1 mM of dNTPs, 1x PCR buffer and 0.5 U Genie Taq DNA polymerase (Merck, India) on Gene Amp PCR System 9700 (Applied Biosystems, USA) programmed to the following cycling

profile: initial denaturation at 94 °C for 5 min followed by optimized number of cycles of 94 ºC for 30 sec for denaturation, primer specific annealing temperature for 30 sec and 72 ºC for 1 min extension followed by the final extension step of 72 ºC for 8 min. The amplified DNA fragments were electrophoresed on 8% non-denaturing polyacrylamide gels (Sambrook and Russel 2001) [30]. The SSR alleles were visualized by silver staining (Sanguinetti *et al.* 1994) [31] and gels were documented using Cano Scan FB1210U flatbed Scanner (Cannon, China). Allele sizes were estimated by comparing the bands generated to the pBR322 DNA-MspI digest ladder (New England Bio Labs, USA).

Data analysis

Principle coordinate analysis (PCoA) using cultural, morphological and pathogenicity characters and Mantel test were carried out using XLSTAT trial version. Clear, intense amplified fragments (markers) of RAPD and SSR were scored and normalized into a data pattern of binary matrix with "1" for presence and "0" for absence. Dice dissimilarity coefficients for molecular markers and mean Euclidean distance for phenotypic and combined marker systems were computed using DARwin ver. 5.0.158 (Perrier and Jacquemoud-Collet, 2006). Hierarchical clustering was also carried out to construct the dendrograms using coefficients by unweighted pair group method with arithmetic mean (UPGMA) using the same program. The software POPGENE (ver. 1.32) was used for statistical analysis of standard population genetics (Yeh *et al*. 1999) [40]. Number of alleles (Na), effective Number of alleles (Ne), total Heterozygosity (Ht), Nei's Heterozygosity (H) and percent polymorphic loci were estimated. Nei's original measures of genetic identity and distances were estimated (Nei 1973). Genotypic diversity was calculated by Shannon's information index (I). Association between markers with pathogenicity of the isolates on the host plant was estimated through stepwise Multiple Regression Analysis (MRA) using SPSS software (SPSS 16.0 Version).

Results

Cultural and morphological characterization

The *M. phaseolina* isolates exhibited high variability in colony characteristics on PDA media. The growth of aerial mycelia was luxuriant and moderately luxuriant among 69% and 31% of the isolates respectively. Mycelial texture was either fluffy (69%) or fibrous (31%). Colony colour of *M. phaseolina* isolates varied from grey to black. The pycnidium (fruiting body) shape varied from round to ovoid and also showed variation in abundance. The conidia length varied from 17.3 – 30.46 µm, while the conidial width ranged from 9.67 – 14.5µm (Supplementary Table 1).

RAPD analysis

A total of 501 markers ranging between 500 and 3000 bp were generated among the isolates by PCR amplification using 44 RAPD primers (Supplementary Table2), out which 499 (99.60%) were polymorphic. Most of the primers showed 100% marker polymorphism except two primers *viz*., OPR-18 (91.66%) and OPG-18(90%). The number of polymorphic marker generated by a primer ranged from 4 (OPM-19) to 18 (OPO-10) with an average of 11.34. Dice dissimilarity coefficients calculated based marker data ranged from 0.233 – 0.751 with an average of 0.543. The maximum dissimilarity of 0.751 was observed between the fungal isolates, MP-29 and MP-6. The least dissimilarity of 0.233 was recorded between the isolates MP-25 and MP-26. UPGMA clustering based on RAPD marker dissimilarity resolved into 6 major groups. Group I comprised of 21 isolates representing from all the geographical regions of collection. Group II &III comprised of 2 isolates each representing from Karnataka. Group IV comprised of 7 isolates representing 4 from Karnataka and 3 from Andhra Pradesh. Group V comprised of 2 isolates representing 1 each from Karnataka and Andhra Pradesh. MP-14 with distinct identity formed an independent group VI.

SSR analysis

Profiling of *M. phaseolina* isolates using 25 SSR primers (F/R) primers (Supplementary Table 3) generated a total of 128 alleles. Among SSR markers, 24 loci (96.0%) were polymorphic. The overall allelic size ranged from 82 – 500 bp and the number of varied from 1 – 9. Dice dissimilarity coefficient based SSR marker data ranged from 0.079 to 0.836 with an average of 0.412. The isolates MP-2 and MP-4 showed least genetic distance (0.132) whereas MP-20 and MP-8 were most divergent (0.836). Parameters of genetic diversity among the isolates were calculated based on allelic data showed that mean value of Na, Ne, and I were 5.000, 2.350 and 0.992 respectively. The Mean value of Ht (0.510) and H (0.502) were also computed (Supplementary Table 3). The Neil's genetic identity ranged from 0.202–0.897 and the genetic distance ranged from 0.108–0.856. The maximum genetic identity (0.897) was recorded between the MP-3 and MP-35. The minimum genetic identity (0.202) was observed between MP-1 and MP-21. However, maximum genetic distance (0.856) was observed between MP-1 and MP-21 and the minimum (0.108) was between MP-35 and MP-3. UPGMA clustering based on SSR marker dissimilarity resolved into 3 major groups. Group I comprised of twenty-five isolates representing from all geographical locations. Group II comprised of seven isolates representing four isolates from Karnataka and three isolates from Andhra Pradesh. Likewise, Group III comprised of three isolates representing two from Tamil Nadu and one from Karnataka.

Mantel test of distance matrices based both molecular markers (RAPD and SSR) was computed. The correlation of distance matrices was found to be positive and significant ($r=0.561$, $p<0.001$).

Assessment of pathogenicity

Morphologically similar isolates behaved differently in respect of their pathogenic reaction on V-1 variety. Mean disease symptoms *viz*., plant survival, wilting and rotting of roots (range 0–100%). Isolates differed significantly in their pathogenic behavior and were categorized into virulent, moderately virulent and avirulent based on percentage of root rot induced on 90 DAI. Majority of the isolates were virulent (45.8%) and moderately virulent (45.8%). Lesser numbers of isolates were avirulent (8.4%). No specific correlation was observed between the morphological characters and pathogenic behavior of the isolates. Total of four isolates were most virulent and showed 100% root rot infection in all the replicates on 90 DAI *viz*., MP-1, MP-5, MP-7 and MP-10. However, MP-5 showed disease symptoms in early days i.e., wilting and death of the plant. Whole root tissue was infected and decayed in all the four isolates on 90 DAI. Based on mean Euclidian distance matrix computed from phenotypic and pathogenic characteristics showed the distances ranged

from 0.874–50.040 with an average of 18.209. PCoA based on phenotypic markers and pathogenicity of isolates resulted in the scatter plot which revealed seven groups. Group I, II, III and IV were major groups representing isolates from all geographical locations. Other three groups were distinct and divergent consisting of isolate *viz*., MP-3, MP-18 and MP-29 (Figure 1). UPGMA based on mean Euclidian distance matrix based on phenotypic and pathogenic reaction showed three major clusters. These major groups represented isolates from all geographical locations.

Marker-trait association

Stepwise MRA was attempted to establish the correlation with cultural, morphological and molecular markers with root rot disease phenotype (Table 2). A total of nineteen markers (independent variables) were correlated with root rot (%) infection. In this, fifteen were RAPDs, three were SSRs and one was associated with phenotypic marker. Out of them, ten markers showed positive and significant correlation (Supplementary Table 4). Standardized coefficient beta value for OPO-063000 was highest (0.750) and lowest was OPD-03831 (0.051). In case of root rot infection by the pathogen, the marker OPI-021656 recorded a R2 value of 36.6% and attained 100% in association with other eighteen markers as indicated in the Table 3.

Similarly, nineteen markers were associated with the wilting (%) of the plant due to the disease. In this, fourteen were RAPDs and five were SSRs. In case of wilting, out of the nineteen markers, seven showed positive with significant correlation. In Wilting, standardized coefficient beta values for OPH-04947 was highest (0.639) and lowest was in OPK-17831 (0.048). In case of wilting, the marker OPI-091971 recorded a R2 value of 32.0% and attained 100% in association with other eighteen markers as indicated in the Table 2.

In case of plant survival against the pathogen, maximum markers showed significant negative correlation. Total eighteen markers were associated with plant survival. Out of eighteen markers, only four markers showed positive with low Standardized coefficient beta value. The Table 2 provides a summary of stepwise MRA for association of marker with the pathogenicity.

Genetic diversity analysis based on phenotype and genotype

The correlation of distance matrices based on phenotypic and genotypic marker systems was found to be positive and significant with less r value by Mantel test (r=0.068, $p<0.015$). Analysis of divergence and inter relationship among *M. phaseolina* isolates were estimated based on the mean Euclidean distance matrix calculated from phenotypic, pathogenic and molecular markers showed distance values ranging from 12.108–174.180 with an average of 69.502. Dissimilarity coefficients estimated based on all marker data revealed that the isolates MP-5 and MP-7were closely related with a value of 12.108. MP-8 and MP-10 were the most divergent with Euclidian distance value of 174.180. The dendrogram generated using all marker data revealed the relationship among isolates from different regions. Clustering based UPGMA method resolved into 4 major groups depicting the inter relationship among isolates (Figure 2). Group I and II were the major groups comprised of isolates representing from all geographical locations. Group III comprised of three isolates representing two from Andhra Pradesh and one from Karnataka. Group IV comprised of four isolates representing two from Andhra Pradesh and one each from Karnataka and Tamil Nadu.

Discussion

M. phaseolina has been found to grow in hot, nutrient-poor soils in various cropping systems in southern India (Andrea *et al.* 2013) [3]. Some studies have also recognized that drought promotes pathogen growth and disease development during the cultivation of many important crops (Almeida *et al.* 2008) [2]. Few studies have reported occurrences of charcoal root rot in specific silk-producing areas in Karnataka, Tamil Nadu and Andhra Pradesh (Philip *et al.* 1992; Marimuthu 2005; Chowdary 2006) [27, 20, 11]. We report a comprehensive survey covering all major silkworm regions of South India, report on the prevalence of diseases caused by *M. phaseolina* in mulberry fields, followed by detailed characterization of the isolates and their susceptibility. We report their pathogenesis in one test cultivar. In recent years, there has been a movement to promote international high quality bivolatine silk. Sericulture is increasing on the road to non-traditional areas of Tamil Nadu and Andhra Pradesh. The frequency and incidence of root rot have increased significantly in these new locations due to intensive cultivation of high-yielding mulberry on degraded, dry and nutrient-deficient soils. A multistate study of *M. phaseolina* root rot incidence and disease intensity in mulberry was conducted. Tamil Nadu has the highest incidence of the disease, followed by Andhra Pradesh, Karnataka and Telangana. Entire mulberry orchards were destroyed in the Gobicettipalayam region of Tamil Nadu. Similar findings have been previously reported by other workers in the neighboring state of Kerala (Ganeshamoorthi *et al.* 2010) [15]. There, sericulture is currently very limited and does not contribute significantly to the country's silk production. There were early plantations established in soil with a history of disease, growing crops such as chiles and tomatoes. Infected host tissues containing conidia can continue to produce conidial inoculum for secondary seeding.

Pycnidia or microsclerotia are extremely difficult to eliminate because they can persist in soil for 2–15 years or longer in root debris (Baird *et al.* 2003; Arora and Pareek 2013; Islam *et al.* 2012) [8, 5, 16]. V-1 is a high yielding variety that has around 70% coverage in the southern sericultural regions. It is qualitatively superior, highly responsive to large inputs like irrigation, and performs remarkably well under these circumstances. But root rot infections were discovered to be prone to this variant (Naik and Sharma 2003) [24]. As a result, incorporating resistance to charcoal root rot into the genetic improvement programme of V-1 is a significant challenge for breeders and pathologists. Since there are no recombination events during multiplication when resistance genes are added to a clonally propagated crop like the mulberry, the trait can be maintained for a longer time and is therefore technically more advantageous. The *M. phaseolina* isolates showed variation in their physical and cultural traits. Conidia size and shape, as well as pyrcinidial abundance, varied greatly. It is feasible to state that parasexualism can result in heterokaryons, which add to the variation, by fusing cells from various hyphae (Almeida *et al*. 2003) [1]. Different farming systems have created the possibility of differentiation, which could result in increased genetic diversity and altered pathogenic behaviour. This could explain the variation in physical characteristics between isolates (Su *et al.* 2001). However, there are a finite number of cultural

and morphological markers, and they are also influenced by environmental factors, such as the nutritional media on which they are *in vitro* cultivated. The abundance and neutrality of molecular markers, such as microsatellites, SSRs, and RAPDs, make them the best choice for measuring genetic variation and evaluating diversification (Su *et al.* 2001; Almeida *et al.* 2003) [44, 1]. Dalziel *et al*. (2009) [12] had recommended a mechanistic analysis of molecular markers in relation to gene function as a more effective way to understand environmental fitness. In our study, RAPD-PCR amplification of *M. phaseolina* isolates resulted in 501 markers of which a very large proportion of them (499, i.e., 99.6%) were polymorphic. Interestingly, most of the primers showed 100% marker polymorphism except only two primers *viz*.,OPR-18 (91.66%) and OPG-18 (90%) indicating high genetic variation. Out of the 25 SSR marker loci analyzed, 24 (96.0%) were polymorphic, reinforcing the high genetic variability as estimated by random markers. Even though, RAPDs are dominant markers with questions raised on their reproducibility but, are useful in generation of quick and cost effective genetic information on organism of lesser economic importance or where no genomic research work has been undertaken. If necessary, random markers can be changed into codominant markers, such as the sequence characterised amplification region (SCAR), to allay worries about the reproducibility of the results. When using random markers, the isolates dice dissimilarity coefficients ranged from 0.233 to 0.751, while SSRs revealed nearly equal genetic distance measurements, ranging from 0.079 to 0.836. SSR, however, could foresee greater genetic diversity with fewer markers. The overall allelic size ranged from 82 to 500 bp, and there were 1 to 9 different alleles. These findings suggest that parasexuality must be taken into account as a potential mechanism for genetic diversity among pathogen isolates (Purukayastha *et al.* 2008) [45]. Using a codominant marker system, Shannon's Information Index (I) was estimated to be greater (0.992). SSRs were also more informative to reveal higher Ht (0.510). These results suggest that *M. phaseolina* isolates are quite heterogenous. Although a teleomorph of *M. phaseolina* is not known, heterogenicity was observed in an apparently solely asexually reproducing species (Tancic *et al.* 2012) [46]. Anyway, an in-depth study is necessary to pinpoint the possible mechanism genetic variation in M. phaseolina isolates. Mantel test of distance matrices based on both molecular marker measures indicated positive correlation with high r value. This indicates that both marker systems are informative to reveal genetics of pathogen as well as host-pathogen interaction in disease development and likely to be useful in breeding for disease resistance. Infectivity of *M. phaseolina* isolates on V-1 mulberry plants showed various disease symptoms. Maximum wilting of plants (100%) was observed by infection with four isolates: Tigulahusahalli (MP-1), Peddavaram (MP-5), Parigi (MP-7) and Vellivalasa (MP-10). rice field. These four isolates also caused 100% root system rot, resulting in plant death in all replicates. The majority of cellulolytic microorganisms, such as *M. phaseolina*, hydrolyze plant cell walls even after plant death. Microsclerotia are released into the soil when the host tissue begins to degrade (Arora and Pareek 2013) [5]. Plant survival showed a similar trend, with no plants surviving due to disease after inoculation with MP-1, MP-5, MP-7 and MP-10 isolates. An isolate (MP-3) from Konthalapalli was pathogenic to V-1 plants (86.6% rot infection), but the isolate was collected from a healthy mulberry orchard. The parasitic fitness of a facultative soil-borne pathogen prior to host invasion depends on its ability to survive, its ability to utilize organic sources, and its ability to eventually colonize host rhizomes by competing with other nearby microorganisms. (Khan 2007) [18]. In general, drought stress is a favorable condition for pathogens to infect hosts, during which transpiration rate, water potential, osmotic potential, turgor potential, and relative water content of plants decrease (Mayek-Parez *et al.* 1997) [21]. In three isolates, namely Parigi (MP-8), Parigi (MP-9), and Devadoddi (MP-18), no discernible wilting or rotting was observed at 90 DAI, indicating that they are nontoxic. Suggests. As expected, 100% viable plants were observed at 90 DAI for these three isolates. The regenerative ability of clonally propagated mulberry to generate new shoots from healthy parts of the shoot or root region results in high plant survival. There seems to be resistance to *M. Phaseolina* infection is controlled by many host genes with little individual influence. On the other hand, growth environment and host conditions also play important roles in disease manifestation (Das *et al*. 2008) [13].

In this study, stepwise MRA allowed us to identify 19 markers (independent variables) that correlated with root rot infection (%). 15 were RAPD, 3 were SSR markers and 1 was a phenotypic marker. Of these, 10 markers showed positive standardized beta coefficients. This explains the high virulence associated with the isolate. This set of 10 markers was able to explain all the variation in the dependent variable. Marker OPI-021656 explained the variation ($R2 = 0.366$) and the association with the SSR marker namely Stv_Mph641a-184. The R2 value increased to 0.999. By adding other relevant markers, the model can account for the dependent variable's contribution to total variability. H. Corruption (%). Similarly, 19 markers were associated with plant wilting (%) due to disease. Among them were 14 his RAPD markers and 5 SSR markers. In this case, 7 out of 19 markers were positive and significantly correlated. As reported in Table 2, marker OPI-091971 recorded an R2 value of 32.0% and achieved 100% in association with 18 other markers. For plant survival to pathogens, the maximal marker showed a significant negative correlation. A total of 18 markers have been associated with plant survival. Of those, only four markers were positive with low standardized coefficient beta. The Mantel test was positive and the r-value of the distance matrix between the phenotypic and genotypic marker systems was low and significant ($r=0.068$, $p<0.015$). Among the phenotypic markers, only hyphal growth was found to be associated with spoilage (%) in MRA analysis. Higher mycelial growth may directly contribute to increased rot in the host plant's root system, with a direct association confirmed. The relatively small number of SSR loci used in correlation studies may explain why we do not identify a large number of SSRs associated with disease symptom decline and atrophy (Virk *et al.* 1996) [36]. Also, the large number of analyzed random markers and the potential for complete genome coverage, as a result, increase the likelihood of establishing trait associations. However, there is no information on the genomic distribution of the SSR markers used in this study, which supersedes published studies (Arias 2011; Bogale *et al*. 2005; Baird *et al.* 2009; Mwang'ombe *et al.* 2008; Bahar and Shahab 2012; Datta and Lal 2013) [4, 9, 7, 23, 6, 14] Factors such as immigration, climatic conditions, different cultural patterns, use of different genotypes in different hosts, high selection pressure, and breeding systems have been used to explain the high genetic diversity of fungi.

(Almeida *et al.* 2008; Purkayastha *et al.* 2008) [2, 28]. The present study sheds light on the genetic underpinnings of these potential diagnostic markers and provides further insight into the molecular evolution of charlotte pathogens. Moreover, isolate grouping did not correlate with geographic origin or pathogenicity in any of the analyses. The sampling strategy used in this study was probably sufficient to detect genetic diversity maximally. Both RAPD and SSR analyzes were very helpful in assessing the intra specific diversity of this pathogen. These results will not only contribute to our understanding of pathogen-induced diseases and improve plant productivity, but also help develop integrated strategies for disease management and breeding programs.

Charcoal root rot caused by *M. phaseolina* is one of the most destructive pathogens affecting mulberry and many other economically important crops. The fungus can deplete young and old mulberry trees and can wipe out entire plantations if control measures are not taken in time. Chemical and biological control measures are ineffective due to the inherent problems associated with soilborne fungi, so breeding for resistance in host plants is the only sustainable way to address this problem. Pathogen genetic diversity and diversity are important aspects that need to be assessed before initiating a charcoal rot resistance breeding program. Pathogenic diversity is a key parameter for breeding durable resistance. We conclude that the genetic variability and diversity of fungi associated with mulberry charcoal root rot is high and therefore a systematic approach is required for breeding. Our study also provided insight into genomic regions that contribute to fungal virulence. The results of this study may have important implications for developing strategies and mining disease resistance genes for breeding from large host plant gene pools. Major and minor genes contributing to resistance traits can be localized by linkage disequilibrium mapping and subsequent pyramiding of the maximum number of alleles for sustainable breed development programs against disease.

Table 1: List of isolates of *M. phaseolina* collected from root rot infested mulberry gardens of South India

Sl. No.	Code	State	Place of collection	Year of collection	Soil type	Age of the plantation in Years	Variety	Longitude and Latitude
1	MP- 1	Karnataka	Tigulahusahalli, Kanakapura	2012	Black	7	V-1	12° 55′ N, 77° 42′ E
2	MP- 2	Telangana	Suryapeta, Nalgonda	2014	Red Sandy	4	V-1	17°13′ N, 79° 63′ E
3	MP- 3	Andhra Pradesh	Konthalapalli, Vijayawada	2012	Black	1	V-1	16° 60′ N, 80° 72′ E
4	MP- 4	Andhra Pradesh	Rangapuram, Vijayawada	2012	Black	1	V-1	16° 60′ N, 80° 72′ E
5	MP- 5	Andhra Pradesh	Peddavaram, Vijayawada	2012	Black	4	V-1	16° 60′ N, 80° 72′ E
6	MP- 6	Andhra Pradesh	Karyapalli, Rayachoti	2013	Red	1	V-1	14° 05′ N, 48° 75′ E
7	MP- 7	Andhra Pradesh	Parigi, Ananthapur	2013	Red	5	V-1	14° 67′ N, 77° 59′ E
8	MP- 8	Andhra Pradesh	Parigi, Ananthapur	2013	Red	2	V-1	14° 67′ N, 77° 59′ E
9	MP- 9	Andhra Pradesh	Parigi, Ananthapur	2013	Red	3	V-1	14° 67′ N, 77°59′ E
10	MP- 10	Tamil Nadu	Vellivalasa, Gobichettipalyam	2013	Red sandy	2	MR-2	12° 82′N, 77° 68′ E
11	MP- 11	Tamil Nadu	Palapalyam, Gobichettipalyam	2013	Red loamy	7.5	V-1	12° 82′ N, 77° 68′ E
12	MP- 12	Tamil Nadu	Govindagrahara, Hosur	2013	Black	5	V-1	12° 74′ N, 77° 82′ E
13	MP- 13	Tamil Nadu	Govindagrahara, Hosur	2013	Black	5	V-1	12° 74′ N, 77° 82′ E
14	MP- 14	Tamil Nadu	Govindagrahara, Hosur	2013	Black	5	V-1	12° 74′ N, 77° 82′ E
15	MP- 15	Tamil Nadu	Kalyanahalli, Hosur	2013	Red loamy	7.5	V-1	12° 74′ N, 77° 82′ E
16	MP- 16	Tamil Nadu	Muthanellore, Hosur	2013	Black	4	V-1	12° 58′ N, 77° 38′ E
17	MP- 17	Karnataka	Devadoddi, Ramnagara	2013	Red loamy	2	V-1	12° 70′ N 77° 28′ E
18	MP- 18	Karnataka	Devadoddi, Rama nagara	2013	Red loamy	1	V-1	12° 70′ N 77° 28′ E
19	MP- 19	Karnataka	S. R. S. Betta, Rama nagara	2013	Red loamy	4	V-1	12° 70′ N 77° 32′ E
20	MP- 20	Karnataka	CSRTI, Mysuru	2013	Red	2	V-1	12° 18′ N 76°38′ E
21	MP- 21	Karnataka	CSRTI, Mysuru	2013	Red	3	V-1	12° 18′ N 76° 38′ E
22	MP- 22	Karnataka	Bapujinagar, Davanagere	2013	Red	4	V-1	17°46′ N 78° 48′ E

23	MP- 23	Karnataka	Bapujinagar, Davanagere	2013	Black	4	V-1	17° 46′ N 78° 48′ E
24	MP- 24	Andhra Pradesh	Iytavaram, Vijayawada	2013	Black	5	V-1	16° 60′ N, 80° 72′ E
25	MP- 25	Andhra Pradesh	Venkatagiri Kota, Chitoor	2014	Red loamy	4	V-1	13° 22′ N 79° 10′ E
26	MP- 26	Karnataka	Kurubarahalli, Mysuru	2014	Red loamy	4	V-1	12° 18′ N 76° 38′ E
27	MP- 27	Karnataka	NagarKotae, Shimoga	2014	Red loamy	4	V-1	13° 92′ N 75° 56′ E
28	MP- 28	Karnataka	Bidar	2014	Red loamy	4	V-1	17° 91′ N 77° 50′ E
29	MP- 29	Andhra Pradesh	Venkatagiri Kota, Chitoor	2014	Red loamy	4	V-1	13° 22′ N 79° 10′ E
30	MP- 30	Karnataka	Bapujinagar, Davanagere	2014	Red loamy	5	V-1	17° 46′ N 78° 48′ E
31	MP- 31	Karnataka	Bogadi, Mysuru	2014	Red loamy	4	V-1	12° 29′ N 76° 59′ E
32	MP- 32	Karnataka	Nanjangud, Mysuru	2014	Red loamy	4	V-1	12° 11′ N 76° 67′ E
33	MP- 33	Karnataka	Nanjangud, Mysuru	2014	Red loamy	4	V-1	12° 11′ N 76° 67′ E
34	MP-34	Telangana	Suryapeta, Nalgonda	2014	Red Sandy	4	V-1	17° 13′ N, 79° 63′ E
35	MP-35	Telangana	Suryapeta, Nalgonda	2014	Red Sandy	3	V-1	17° 13′ N, 79° 63′ E

Table 2: Summary of stepwise MRA for association of markers and pathogenicity

Trait	Marker	R^2	Adjusted R^2	F Change	p value of F change
Root rot	OPI-02$_{1656}$	0.366	0.347	19.070	0.000
	LAS 21& 22$_{398}$	0.570	0.543	15.152	0.000
	OPD-20$_{628}$	0.670	0.638	9.431	0.004
	StvMph_247a$_{167}$	0.755	0.722	10.398	0.003
	Mycelia Growth	0.811	0.779	8.672	0.006
	OPO-06$_{3000}$	0.863	0.834	10.607	0.003
	OPN-10$_{2427}$	0.896	0.869	8.398	0.007
	OPM-04$_{1375}$	0.920	0.895	7.804	0.010
	OPA-18$_{1375}$	0.941	0.920	9.055	0.006
	OPN-20$_{600}$	0.957	0.939	8.994	0.006
	OPH-04$_{1741}$	0.968	0.953	8.272	0.009
	OPH-12$_{2024}$	0.979	0.967	10.721	0.003
	OPO-10$_{1027}$	0.984	0.974	7.146	0.014
	OPN-09$_{2024}$	0.989	0.982	9.417	0.006
	OPAA-14$_{564}$	0.993	0.988	11.382	0.003
	OPK-18$_{1904}$	0.996	0.992	9.613	0.006
	OPO-06$_{987}$	0.998	0.995	13.989	0.002
	OPD-03$_{831}$	0.998	0.997	8.929	0.009
	StvMph_641a$_{184}$	0.999	0.998	12.168	0.003
Leaf wilting	OPI-09$_{1971}$	0.320	0.299	15.536	0.000
	OPK-17$_{831}$	0.503	0.472	11.743	0.002
	OPAC-20$_{689}$	0.678	0.646	16.817	0.000
	OPH-04$_{947}$	0.772	0.742	12.517	0.001
	OPD-05$_{947}$	0.841	0.814	12.613	0.001
	OPJ-20$_{1059}$	0.885	0.860	10.484	0.003
	StvMph_99a$_{136}$	0.916	0.894	9.974	0.004
	StvMph_273a$_{180}$	0.938	0.918	9.098	0.006
	OPJ-09$_{947}$	0.953	0.936	8.329	0.008
	OPC-14$_{1832}$	0.965	0.950	8.085	0.009
	OPC-14$_{643}$	0.977	0.965	11.483	0.003
	MP-32$_{168}$	0.984	0.975	9.456	0.006
	OPAA-19$_{1100}$	0.989	0.982	10.617	0.004
	OPD-03$_{1584}$	0.992	0.987	8.085	0.010
	OPO-10$_{1904}$	0.995	0.991	8.671	0.008
	StvMph_641a$_{184}$	0.997	0.994	11.510	0.003
	OPI1-09$_{1828}$	0.998	0.996	10.200	0.005
	SSR-10$_{134}$	0.999	0.998	13.164	0.002
	OPA-03$_{2216}$	0.999	0.998	8.630	0.010

Plant survival	OPN-20$_{600}$	0.354	0.334	18.059	0.000
	OPD-20$_{628}$	0.551	0.523	14.089	0.001
	OPAA-14$_{831}$	0.675	0.643	11.771	0.002
	OPAA-19$_{1375}$	0.748	0.715	8.741	0.006
	MP-11$_{194}$	0.826	0.796	13.057	0.001
	OPAA-14$_{564}$	0.870	0.842	9.481	0.005
	OPH-11$_{1050}$	0.897	0.870	7.059	0.013
	OPA-18$_{1375}$	0.921	0.897	8.006	0.009
	OPH-4$_{3000}$	0.945	0.925	10.539	0.003
	OPI-02$_{831}$	0.967	0.953	15.921	0.001
	OPQ-20$_{1816}$	0.977	0.965	9.743	0.005
	OPA-03$_{602}$	0.982	0.972	6.125	0.022
	OPN-20$_{1450}$	0.986	0.977	6.173	0.021
	OPH-04$_{2024}$	0.991	0.986	13.172	0.002
	OPC-14$_{2473}$	0.995	0.991	13.559	0.002
	OPAA-19$_{2200}$	0.998	0.996	19.728	0.000
	OPN-20$_{831}$	0.999	0.997	11.888	0.003
	OPN-10$_{2027}$	1.000	0.999	31.444	0.000

Supplementary Table 1: Cultural and morphological variability among the isolates of *M. phaseolina*

Sl. No.	Code	Mycelial growth	Mycelial texture	Colony colour	Mean colony dia. (mm) (2DAI)	Mean conidial length (µm)	Conidial width (µm)	Pcnydial abundance	Pcynidial shape
1	MP- 1	Moderate luxuriant	Fibrous	Greyish black	75.8	20.95	14.5	Low	Oblong
2	MP- 2	Luxuriant	Fluffy	Greyish white	65.375	24.04	12.5	Medium	Round
3	MP- 3	Moderate luxuriant	Fibrous	Greyish black	71.25	30.46	14.02	Low	Oblong
4	MP- 4	Luxuriant	Fluffy	Greyish white	64.37	24.17	14.02	High	Oblong
5	MP- 5	Luxuriant	Fluffy	Greyish white	74.5	18.3	14.5	High	Round
6	MP- 6	Luxuriant	Fluffy	Greyish white	75.6	28.04	14.5	Medium	Round
7	MP- 7	Luxuriant	Fluffy	Greyish black	76.4	22.3	14.5	High	Oblong
8	MP- 8	Luxuriant	Fluffy	Greyish black	71.3	21.8	9.67	Medium	Round
9	MP- 9	Luxuriant	Fluffy	Greyish white	79.16	18.85	9.18	Medium	Round
10	MP- 10	Luxuriant	Fluffy	Greyish white	77	28.04	14.5	Low	Oblong
11	MP- 11	Luxuriant	Fluffy	Greyish black	66.12	24.17	14.5	High	Round
12	MP- 12	Luxuriant	Fluffy	Greyish black	76.375	17.3	14.5	Low	Round
13	MP- 13	Moderate luxuriant	Fibrous	Greyish black	69.25	29.01	9.67	Low	Oblong
14	MP- 14	Luxuriant	Fluffy	Greyish black	76.37	18.5	14.5	Medium	Round
15	MP- 15	Luxuriant	Fluffy	Greyish white	76.75	18.85	9.18	Medium	Round
16	MP- 16	Luxuriant	Fluffy	Greyish black	61.5	26.59	9.67	High	Oblong
17	MP- 17	Luxuriant	Fluffy	Greyish white	74.5	24.5	14.5	High	Round
18	MP- 18	Luxuriant	Fluffy	Greyish white	72	28.04	14.5	High	Oblong
19	MP- 19	Luxuriant	Fluffy	Greyish white	69.5	18.80	14.5	Low	Round
20	MP- 20	Moderate luxuriant	Fibrous	Greyish black	74.5	21.4	14.5	Medium	Oblong
21	MP- 21	Luxuriant	Fluffy	Greyish black	66.5	24.17	19.34	Medium	Round
22	MP- 22	Luxuriant	Fluffy	Greyish black	63.5	28.04	14.5	High	Oblong
23	MP- 23	Luxuriant	Fluffy	Greyishwhite	66.4	28.04	14.5	Medium	Round
24	MP- 24	Luxuriant	Fluffy	Greyish white	72	28.04	14.5	High	Oblong
25	MP- 25	Luxuriant	Fluffy	Greyish black	72	28.04	14.5	High	Oblong
26	MP- 26	Luxuriant	Fluffy	Greyish white	64.37	24.17	14.02	High	Oblong
27	MP- 27	Luxuriant	Fluffy	Greyish black	72	28.04	14.5	High	Oblong
28	MP- 28	Luxuriant	Fluffy	Greyish black	66.7	18.80	14.5	Low	Round
29	MP- 29	Luxuriant	Fluffy	Greyish black	75.8	28.04	14.5	High	Oblong
30	MP- 30	Luxuriant	Fluffy	Greyish black	71.25	28.04	14.5	High	Oblong
31	MP- 31	Luxuriant	Fluffy	Greyish black	65.375	28.04	14.5	High	Oblong
32	MP- 32	Luxuriant	Fluffy	Greyish black	63.9	18.80	14.5	High	Oblong
33	MP- 33	Luxuriant	Fluffy	Greyish black	65.9	28.04	14.5	High	Oblong
34	MP-34	Luxuriant	Fluffy	Greyish black	76.12	28.04	14.5	High	Oblong
35	MP-35	Luxuriant	Fluffy	Greyish black	75.8	28.04	14.5	High	Oblong

Supplementary Table 2: List of RAPD primers used in the characterization of *M. phaseolina* isolates and marker polymorphism

Sl. No.	Primer	Sequence '5------------3'	Total Markers	No of Polymorphism markers	Polymorphism (%)
1	OPD-13	GGGGTGACGA	14	14	100
2	OPN-12	CACAGACACC	13	13	100
3	OPH-4	CACAGACACC	15	15	100
4	OPH-12	ACGCGCATGT	16	16	100
5	OPN-20	GGTGCTCCGT	15	15	100
6	OPO-6	CCACGGGAAG	14	14	100
7	OPO-10	TCAGAGCGCC	18	18	100

8	OPN-10	ACAACTGGGG	16	16	100
9	OPH-11	CTTCCGCAGT	9	9	100
10	OPAA-18	TGGTCCAGCC	12	12	100
11	OPJ-9	TGAGCCTCAC	11	11	100
12	OPN-17	CATTGGGGAG	8	8	100
13	OPAA-16	GGAACCCACA	11	11	100
14	OPK-17	CCCAGCTGTG	12	12	100
15	OPAA-14	AACGGGCCAA	14	14	100
16	OPN-9	TGCCGGCTTG	11	11	100
17	OPN-7	CAGCCCAGAG	12	12	100
18	OPH-3	AGACGTCCAC	13	13	100
19	OPAA-19	TGAGGCGTGT	11	11	100
20	OPK-18	CCTAGTCGAG	11	11	100
21	OPD-20	ACCCGGTCAC	12	12	100
22	OPJ-20	AAGCGGCCTC	12	12	100
23	OPI-19	AATGCGGGAG	10	10	100
24	OPQ-20	TCGCCCAGTC	11	11	100
25	OPI-9	TGGAGAGCAG	11	11	100
26	OPI-2	GGAGGAGAGG	9	9	100
27	OPM-9	GTCTTGCGGA	11	11	100
28	OPG-17	ACGACCGACA	12	12	100
29	OPC-15	GACGGATCAG	10	10	100
30	OPR-15	GGACAACGAG	11	11	100
31	OPA-18	AGGTGACCGT	10	10	100
32	OPD-18	GAGAGCCAAC	14	14	100
33	OPC-14	TGCGTGCTTG	10	10	100
34	OPD-13	GGGGTGACGA	8	8	100
35	OPAA-14	AACGGGCCAA	9	9	100
36	OPG-18	GGCTCATGTG	10	9	90
37	OPAC-20	ACGGAAGTGG	9	9	100
38	OPD-3	GTCGCCGTCA	11	11	100
39	OPM-4	GGCGGTTGTC	12	12	100
40	OPR-18	GGCTTTGCCA	12	11	91.66
41	OPA-3	AGTCAGCCAC	12	12	100
42	OPC-3	GGGGGTCTTT	7	7	100
43	OPM-19	CCTTCAGGCA	4	4	100
44	OPD-5	CCTTCAGGCA	9	9	100
		Total	501	499	99.6

Supplementary Table 3: List of SSR primers used in the characterization of *M. phaseolina* isolates and marker polymorphism

Sl. No.	Marker	F/R primer sequence	Allelic size range	Na	Ne	I	Ht	H	Ta	Accession number
1	AF-51	F: GGCATATTGAGTATGGTATGGAT R: GCTC CCG AGA TCT TGT TCA	113-191	5.000	1.158	0.353	0.138	0.136	46	FI11345
2	MP-5	F: GTGCGAGAAAAATCCGTAAAC R: GTGCGAGAAAAATCCGTAAAC	107-179	7.000	2.772	1.302	0.649	0.639	51	
3	SSR-11	F: TATTTCGTGCAAGGACTTGG R: CTTGGTCCCTGGATATGGA	180-252	3.000	1.901	0.724	0.482	0.474	51	—
4	SSR-10	F: CGAGCTAATGGTGGCAGGAT R: AACAACAAAACGGCTCATCG	114-202	5.000	1.389	0.624	0.284	0.280	50	—
5	LAS-15&16	F: GCCAGATCCGTGCCCACTG R: CATGCAGAGGTCGCAAAGTG	312-500	3.000	1.814	0.795	0.455	0.449	58	—
6	LAS-21&22	F: GGAAGATGATGGGATGGTTGC R: GTACAAGAACGAACTCCGGGT	370-398	5.000	3.101	1.290	0.688	0.678	55	—
7	MP-11	F: AGCCCATGAAGTGGAAAGCTC R: GAAAGAGGTAACCCGCGTTGT	94-218	7.000	3.166	1.347	0.694	0.684	58	FI113441
8	MP-29	F: TAGTGCTGAAGCCAGAAG R: CTTAACCTACCCCGATTG	94-124	5.000	1.255	0.485	0.206	0.203	58	FI113459
9	MP-31	F: CTTAACCTACCCCGATTG R: AGTGCTGAAGCCAGAAGTC	82-242	9.000	5.988	1.974	0.847	0.833	58	FI113461
10	MP-35	F: AACGGGAAAAATTAATGACACG R: AGGGCAAAAGAAGTAAGAGTGC	116-120	2.000	1.471	0.500	0.325	0.320	58	FI113465
11	SSR-12	F: AAGCGCCAACAGAGATGACGA R: GACTGCCGAAACACCGAAA	218-390	6.000	1.691	0.886	0.415	0.409	55	—
12	MP-32	F: TAGTGCTGAAGCCAGAAGTC R: CCCACAGAAAGAAGCTTAAC	166-198	8.000	2.615	1.356	0.627	0.618	58	FI113462
13	Stv_Mph-232a	F: ACTGGTAAGCGCATCCTTTCATAC R: TATAAAAAGGTGCAGCGGTGATG	160-261	6.000	2.698	1.276	0.639	0.629	52	GU944024

14	Stv_Mph-254a	F: GGAATGCGAGTCCAAGGTAAATC R: CGTTCTTTGGTTAGCATCCTTTTG	238-345	4.000	1.561	0.707	0.365	0.360	54	GU944046
15	Stv_Mph-368a	F: CTTCCTCGTGCGTACAAGATCAG R:TATGGGATCAGAAGCACATGACTG	140-226	6.000	3.346	1.429	0.712	0.701	52	GU944159
16	Stv_Mph-641a	F: TTCATCATCCCATCACCTACATCTC R: TGGTGATGATGGAAGACGGTG	110-184	7.000	1.963	1.108	0.498	0.491	54	GU944433
17	Stv_Mph-29a	F: TTCGATCATGTGTCAGAGGGTG R: AAGGACATGACGGACAAAGC	170-212	4.000	1.959	0.907	0.498	0.490	56	GU943820
18	Stv_Mph-45a	F: TGCGCTACGTACAAGAAGCTAGAAC R: CGGCAGTTATATGACGCTATTTCC	100	1.000	1.000	0.000	0.000	0.000	52	GU943837
19	Stv_Mph-99a	F: GATAGAAGCGGCTCTGGGTAAAAG R: TAGCGCACGAGCATACTTTCTTC	123-160	4.000	2.346	1.024	0.583	0.574	51	GU943891
20	Stv_Mph-155a	F: ACCATTGGAATTCCGTCATCTTC R: GAACATCAGTGAGGAAAGGAGGAG	130-190	5.000	2.626	1.170	0.628	0.619	50	GU943947
21	Stv_Mph-247a	F: TTTATCCTGTGTTGCGCTGTATTG R: GTTCACTGCTCTGTCTCGGCTTAG	131-217	6.000	2.667	1.302	0.636	0.625	52	GU944039
22	Stv_Mph-273a	F: GATCTCATTTGTTCAGCGAAAAGG R: CCTGTCTCCTGAGAGGGAGGTAG	130-180	4.000	3.022	1.189	0.679	0.669	53	GU944065
23	Stv_Mph-484a	F: ATGGGGTTCGAGAGAAAACATAGG R: GTTAGTGAGATGGTTGAAAACGCC	147-194	4.000	1.792	0.815	0.449	0.442	51	GU944276
24	Stv_Mph-19b	F: GTACTTTTGCATGGCTACACCCC R: ACCTCCCCAACAACCACCAC	160-271	4.000	2.190	0.894	0.551	0.543	52	GU943811
25	Stv_Mph-173	F: TCAAGGCTCATTCAGCATTACAAC R: GGAGAGGAATTCTGATGATGGTG	135-242	5.000	3.282	1.357	0.706	0.695	50	GU943965
			Mean	5.000	2.351	0.992	0.510	0.502		
			St. Dev	1.826	1.034	0.422	0.203	0.199		

Na is the observed number of alleles
Ne is the effective number of alleles [Kimura and Crow (1964)]
I is the Shannon's information index
H is the Neil's (1973) expected Heterozygosity
Ht is the Total Heterozygosity

Supplementary Table 4: Classification of pathogenicity among *M. phaseolina* isolates

State	No. of Virulent Isolates	No. of Moderately Virulent Isolates	No. of Avirulent Isolates
Karnataka	6	11	1
Andhra Pradesh	5	1	2
Telangana	1	1	0
Tamil Nadu	4	3	0
Total	16	16	3

Supplementary table 5: Coefficients for dependent variable in stepwise MRA for association of pathogenicity

Trait	Marker	Unstandardiz-ed coefficient beta	SE	Standardized coefficient beta	t value	P value
Root rot	$OPI\text{-}02_{1656}$	-41.166	0.659	-0.616	-62.444	0.000
	$LAS\ 21\&\ 22_{398}$	-109.166	2.337	-0.651	-46.987	0.000
	$OPD\text{-}20_{628}$	35.604	0.915	0.507	38.933	0.000
	$StvMph_247a_{167}$	-4.316	0.154	-0.229	-27.966	0.000
	Mycelia Growth	-33.666	1.204	-0.381	-27.964	0.000
	$OPO\text{-}06_{3000}$	8.736	0.480	0.750	18.218	0.000
	$OPN\text{-}10_{2427}$	26.709	0.774	0.416	34.520	0.000
	$OPM\text{-}04_{1375}$	-18.469	0.688	-0.297	26.839	0.000
	$OPA\text{-}18_{1375}$	3. 198	0.603	0.503	5.300	0.000
	$OPN\text{-}20_{600}$	13.979	0.906	0.158	15.430	0.000
	$OPH\text{-}04_{1741}$	-17.126	0.850	-0.304	-20.138	0.000
	$OPH\text{-}12_{2024}$	3.894	0.210	0.205	18.499	0.000
	$OPO\text{-}10_{1027}$	9.766	0.853	0.131	11.451	0.000
	$OPN\text{-}09_{2024}$	-3.184	0.289	-0.172	-11.009	0.000
	$OPAA\text{-}14_{564}$	5.187	0.713	0.074	7.271	0.000
	$OPK\text{-}18_{1904}$	5.973	0.720	0.096	8.298	0.000
	$OPO\text{-}06_{987}$	-2.490	0.445	-0.223	-5.600	0.000
	$OPD\text{-}03_{831}$	2.853	0.604	0.051	4.725	0.000
	$StvMph_641a_{184}$	-4.197	1.203	-0.048	-3.488	0.003
Leaf wilting	$OPI\text{-}09_{1971}$	-33.298	0.698	-0.499	-47.725	0.000
	$OPK\text{-}17_{831}$	83.839	2.104	0.048	39.844	0.000
	$OPAC\text{-}20_{689}$	37.224	0.686	0.636	54.263	0.000
	$OPH\text{-}04_{947}$	80.224	1.619	0.639	49.560	0.000
	$OPD\text{-}05_{947}$	-22.083	0.643	-0.377	-34.325	0.000
	$OPJ\text{-}20_{1059}$	-18.732	0.640	-0.310	-29.278	0.000

	StvMph_99a$_{136}$	12.766	0.628	0.217	20.328	0.000
	StvMph_273a$_{180}$	-16.385	1.217	-0.130	-13.460	0.000
	OPJ-09$_{947}$	-9.200	0.761	-0.119	-12.091	0.000
	OPC-14$_{1832}$	-9.923	0.670	-0.167	-14.803	0.000
	OPC-14$_{643}$	8.822	1.109	0.070	7.953	0.000
	MP-32$_{168}$	-26.414	1.737	-0.151	-15.208	0.000
	OPAA-19$_{1100}$	-5.887	0.506	-0.101	-11.632	0.000
	OPD-03$_{1584}$	11.472	1.213	0.110	9.459	0.000
	OPO-10$_{1904}$	-6.521	0.688	-0.098	-9.484	0.000
	StvMph_641a$_{184}$	-6.749	1.117	-0.074	-6.041	0.000
	OPI1-09$_{1828}$	4.921	0.729	0.082	6.752	0.000
	SSR-10$_{134}$	-.533	0.156	-0.046	-3.425	0.004
	OPA-03$_{2216}$	-4.930	1.678	-0.039	-2.938	0.010
Plant survival	OPN-20$_{600}$	-58.829	1.001	-0.538	-58.785	0.000
	OPD-20$_{628}$	-13.900	0.644	-0.160	-21.599	0.000
	OPAA-14$_{831}$	-16.057	0.481	-0.228	-33.366	0.000
	OPAA-19$_{1375}$	-13.330	0.497	-0.191	-26.806	0.000
	MP-11$_{194}$	-86.393	1.418	-0.413	-60.909	0.000
	OPAA-14$_{564}$	-35.612	0.636	-0.409	-55.964	0.000
	OPH-11$_{1050}$	5.677	0.644	0.071	8.819	0.000
	OPA-18$_{1375}$	-16.462	0.543	-0.220	-30.306	0.000
	OPH-4$_{3000}$	53.500	1.478	0.256	36.187	0.000
	OPI-02$_{831}$	-10.123	0.483	-0.135	-20.942	0.000
	OPQ-20$_{1816}$	15.837	0.837	0.159	18.913	0.000
	OPA-03$_{602}$	-14.577	0.831	-0.117	-17.543	0.000
	OPN-20$_{1450}$	-11.107	0.645	-0.120	-17.223	0.000
	OPH-04$_{2024}$	-10.866	1.048	-0.072	-10.371	0.000
	OPC-14$_{2473}$	-5.721	0.525	-0.082	-10.890	0.000
	OPAA-19$_{2200}$	8.622	0.774	0.079	11.132	0.000
	OPN-20$_{831}$	-11.064	1.674	-0.053	-6.611	0.000
	OPN-10$_{2027}$	-3.218	0.574	-0.040	-5.608	0.000

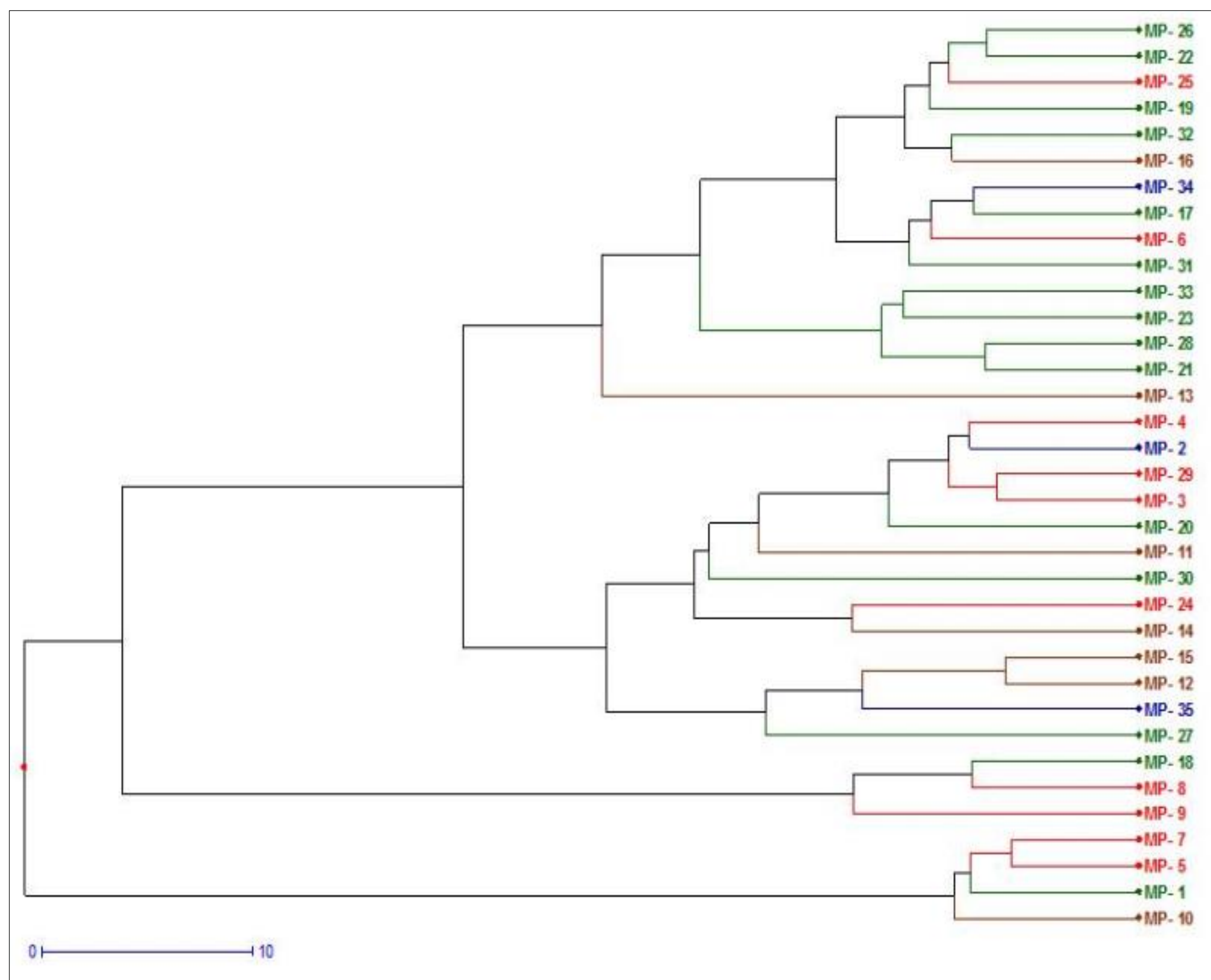

Fig 1: PCOA analysis *M. phaseolina* isolates based on cultural, morphological and pathogenic reaction

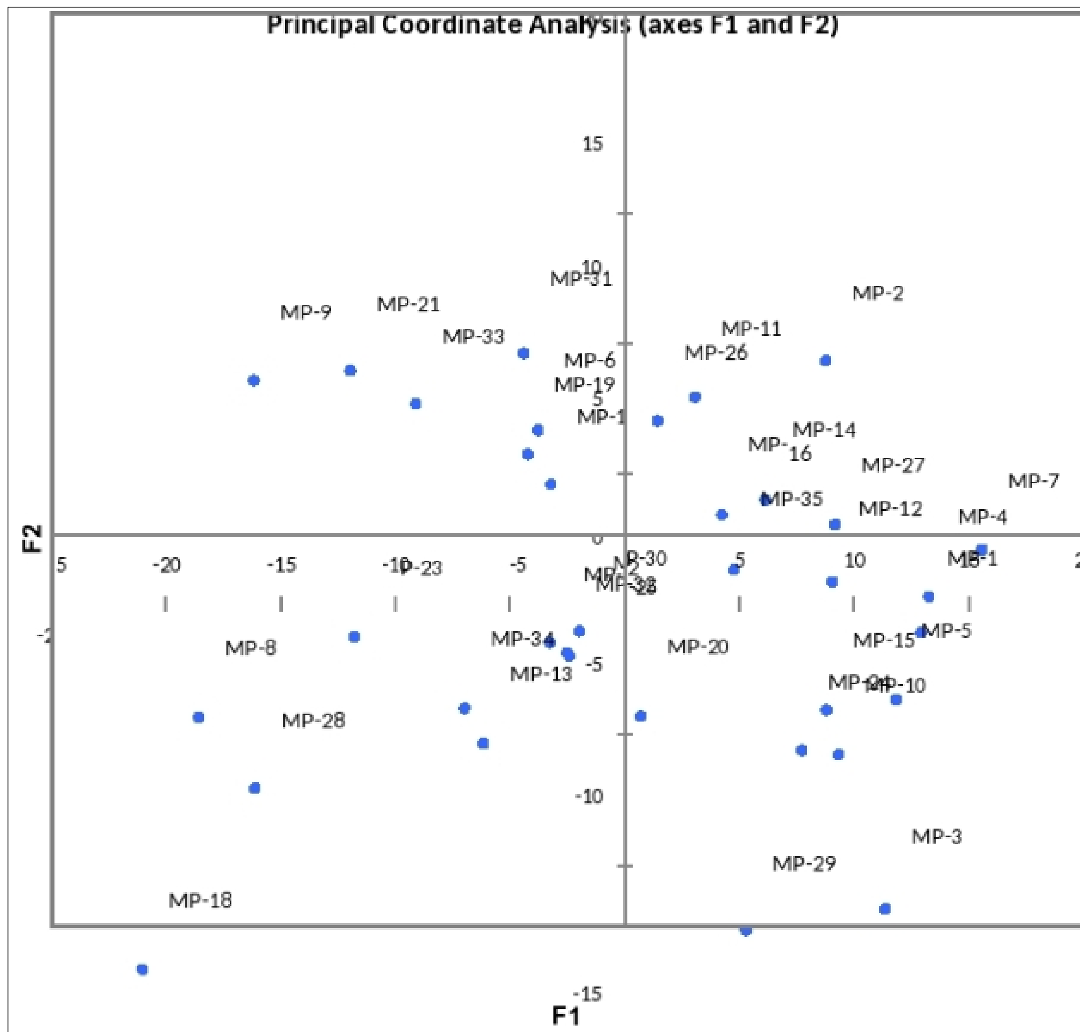

Fig 2: Dendrogram depicting the interrelationship among *M. phaseolina* isolates based on mean Euclidian distance matrix computed from phenotypic, pathogenic and molecular marker data

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