MORPHOLOGICAL AND GENETIC VARIABILITY AND HOST RESISTANCE RESPONSE OF SORGHUM RECOMBINANT INBRED LINES (RILs) TO A VIRULENT ISOLATE OF *Macrophomina Phaseolina* (Tassi) Goid.

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1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) on a world basis represent four per cent of the cereal production. Sorghum ranks fifth, among the cereals after wheat, maize, rice and barley. In India sorghum is grown over an area of 9015.0 hectare with the production of 7650.0 tonnes and productivity of 849 kg/ha. Karnataka has an area of 1672.0 hectare, with a production of 788.0 tonnes and productivity of 471 kg/ha (Anon., 2006). In India sorghum is one of the important cereal crop, well adopted to low input and low moisture situations.

Being an important food crops for larger section of people in Asia and Africa and a good source of fodder for cattle it is also used to produce jaggery, syrup, ethanol (Biofuel), beer, biscuits etc. and it is an agro based industrial crop. Its alternate uses are poultry feed, sugar and alcohol (Anon., 1995).

Sorghum is grown both during Southwest monsoon (*kharif*) and post monsoon (*rabi*) season. *Rabi* crop produces high quality grains as they mature during winter season under clear dry and rain free climate. However, low levels of productivity in *rabi* sorghum is due to drought and is aggravated by charcoal rot disease which usually appears at grain maturity stage and cause severe lodging. Most improved varieties and hybrids are susceptible to the disease.

The loss in grain weight due to charcoal rot in *rabi* cultivars varies from 18.53 to 63.22 per cent (Anon., 1999). A maximum of 70.73 per cent grain weight loss has been reported in CSH13R, a high yielding commercial hybrid (Patil, 1980). The loss in grain yield is always more in *rabi* (40.83%) than in *kharif* (17.69%) (Patil, 1980).

Breeding for charcoal rot resistance began about three decades ago (Rosenow, 1992). Although the inheritance of charcoal rot resistance is complex, relatively good progress has been made in development of screening technique and breeding for higher level of host resistance. Selection for stiff stalk and drought tolerance, specially the non-senescent type (stay green), combined with high yield is considered to be more productive than breeding for charcoal rot resistance alone (Maunder, 1993). Despite efforts, the level of resistance achieved so far is low. Lack in progress of selection is mainly due to low heritability.

An adequate knowledge about the existence of variability in the pathogen would be most important and highly desirable to strengthen breeding attempts in attaining host plant resistance, which alone, can adequately manage the disease.

Variation, is a rule in nature which underlines the competence of an individual to survive and flourish. This "basic thumb rule" for survival, is well understood by an asexual, cosmopolitan, soil borne and a plurivorous pathogen like *Macrophomina phaseolina* (Tassi) Goid. which has evolved to have wide host range to survive by means of asexual way of reproduction in over 75 different families covering more than 500 plant species (Dhingra and Sinclair, 1973b).The greater environmental adoptive flexibility and an increasingly alarming innovativeness in the variety of diseases it produces on different host plants. Understanding the variability in the pathogen, it is necessary to strengthen the breeding strategies for host plant resistance.

Attempts based on molecular technique to develop faster and unambiguous methods for differentiation of *Macrophomina* have been made, using RFLP of rDNA-ITS, RAPD (Su *et al.,* 2001) and AFLP (Vandermark *et al.,* 2000).

Keeping above points in view the following objectives were addressed in the present investigation.

1. Collection, morphological and molecular characterization of different sorghum isolates of *Macrophomina phaseolina*
2. To study the path of movement of *Macrophomina phaseolina* in sorghum
3. To map the variability of charcoal rot component traits to D_5 isolate of *M. phaseolina* among RILs of IS 22380 × E36-1 cross.

2. REVIEW OF LITERATURE

Charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid., is a root and stalk rot disease of great destructive potential in most sorghum growing regions of the world. All improved and high yielding cultivars are susceptible to charcoal rot. Chemical control of charcoal rot is not effective and utilization of host plant resistance is the most realistic alternative for disease management.

All the reviews carried out by earlier workers pertaining to the objectives set up for the present research work has been compailed in this section.

The pathogen

Macrophomina phaseolina, the causal organism of seedling blight and hollow stem of sorghum, was first reported in India during 1931 by Uppal. It is the pycnidial stage of *Rhizoctonia bataticola* (Ashby, 1927, Haigh, 1928 and McRae, 1929) with the following synonyms.

Synonyms:

Macrophoma phaseoli Maubl; *M. corchori* Saw; *M. cajani* Syd and Butl; *M. sesami*, Saw; *Macrophomina philippinensis* Petr. and *Sclerotium bataticola* Taub (Ashby, 1927). Goidanich (1947) examined the original culture of *M. phaseoli* collected by Tassi and renamed it as *Macrophomina phaseolina* (Tassi) Goid. Rhizoctonia is predominantly subterranean in habit, living both as a parasite and saprophyte.

Distribution

The charcoal rot disease caused by, *M. phaseolina*, is one of the most destructive pathogens in arid regions and has a wide host range (Waller, 1976).

In India, it has been reported from Andhra Pradesh, Bihar, Gujarat, Karnataka, Maharashtra, Madhya Pradesh, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal (Uppal, 1931; Likhite and Kulkarni, 1934; Patel *et al.,* 1949; Venkataram, 1950; Thirumalachar, 1955; Chattopadyay and Bhattacharya, 1968; Deshpande *et al.*, 1969 and Phillip *et al.,* 1969). Dry root caused by *R. bataticola* has been reported in Australia, Ethiopia, India, Iran, Lebenon, Syria, Turkey and United States (Nene, 1978). One of the major diseases found in Myanmar was dry root rot caused by *M. phaseolina* (Aung *et al.,* 1992).

Economic importance

Charcoal rot of sorghum caused by *M. phaseolina* has been identified as a destructive disease in post rainy season in Karnataka, Maharashtra, Gujarat and Andhra Pradesh (Anahosur and Patil, 1983, Subramaniam, 1994 and Reddy and Hindumathi, 1997) and in other parts of the world where sorghum is cultivated (Mughogho and Pande, 1984). Its severity is more in susceptible cultivars, when grain filling coincides with periods of moisture stress and high soil temperature (Edmunds, 1962). The loss in grain weight due to the disease in *rabi* cultivars varies from 18.53 to 63.22 per cent (Anon., 1999). Maximum of 70.73 per cent grain weight loss has been reported in CSH13R, a high yielding commercial hybrid (Patil, 1980).

Severe epidemics of charcoal rot of sorghum in Karnataka were reported by various workers (Anahosur and Rao, 1977, Hiremath and Palakshappa, 1994). Patil (1980) reported that loss in grain yield is always more in *rabi* (40.83%) than in *kharif* (17.69%).

Symptoms

Different kinds of symptoms are associated with charcoal rot; root rot, soft stalks, lodging of plants, pre-mature drying of stalks and poorly developed panicles with small inferior quality grain are common (Hsi, 1956 and Uppal *et al.,* 1936). The most striking indication of the disease is lodging of plants as they approach maturity. Lodging is due to the weakened stalk, which results in disintegration of the pith and cortex of basal stem. The lignified fibrovascular bundles remain suspended as separate strands in the hollow stalk; hence, it was also referred as 'hollow stalk of sorghum' by Uppal *et al.* (1936). The vascular bundles are profusely covered with tiny black sclerotia of the pathogen, which give the black charcoal appearance to the affected area. Indeed the name 'charcoal rot' describes the appearance of the disease inside infected roots and stalks.

The disease is also characterized by light brown discoloration of the sub epidermal tissue in the root and lower part of the stem. Infected plants, at first do not show above ground symptoms. However, in advanced stages, leaves turn yellow and wilt, but remain attached to the plant. When the epidermis is removed sclerotia appear as grayish black on the tissue. The sclerotium resembles a sprinkling of fine charcoal powder.

Fungal isolations from diseased root and stalks usually revealed the association of *M. phaseolina* and *Fusarium moniliformae* (Tullis, 1951). Similar observations were also made in India (Mughogho and Pande, 1984). *M. phaseolina* is a root-inhabiting fungus (Garrett, 1956) with little or no saprophytic growth in either soil or on dead parts of infected plants (Norton, 1953, Edmunds *et al.,* 1964). In the absence of host plants, it survived over many seasons as small black sclerotia in diseased root and stem debris or in soil after decay of the plant material (Smith, 1969, Bhattacharya and Samaddar, 1976). Thus, the primary source of inoculum is sclerotia in the soil.

Host range

Fifty nine species have been recorded under genus *Rhizoctonia* (Saksena and Vaartaja, 1961, Yamamoto, 1962 and Hussain and McKeen, 1963). Among them *R. bataticola* is one of the important species, which has a very wide host range and causes root rot,charcoal rot and ashy blight in most of the cereals, pulses, oilseeds, fibres, vegetables, ornamental plants, plantation and horticultural crops (Small, 1925 and 1928; Uppal, 1934; Likhite and Kulkarni, 1934; Hansford, 1943 and Young, 1949).

Singh *et al.* (1990) identified eleven new hosts, in addition to ten species which were identified as new pycnidial hosts. It was concluded that, *M. phaseolina* was prevalent in all commonly cultivated crops in the semi-arid tropics.

Singh and Bhowmix (1991), reported sesamum root rot incidence upto 71.5 per cent in Delhi, Haryana, Uttar Pradesh, Karnataka and Tamil Nadu. Singh (1991) and Maholay (1992) reported stolon rot of *Mentha arvensis* (Linn) and seed and pod rot of butter bean, respectively from Indian Institute of Horticultural Research (IIHR), Karnataka, India.

Thakur *et al.* (1992) and Mathur (1993) have identified *Crocus sativus* and *Cyamopsis senegalensis* as new hosts for *M. phaseolina*.

Viability

Macrophomina phaseolina was known to survive as sclerotia in corn and sorghum stalk residues for 18 and 16 months, respectively. Large number of viable sclerotia were isolated from corn and sorghum stalk in final collections (Cook *et al.,* 1973). *M. phaseolina* is capable of living saprophytically on dead organic tissues particularly on many of its natural hosts producing sclerotial bodies (Sen and Bandopadhyay, 1988). Ecological behaviour and management aspects were exhaustively covered by Sen and Bandopadhyay (1988) and Chandra and Kehri (1997).

Factors affecting disease incidence

Incidence of charcoal rot was reported to be less during *kharif* than *rabi* in two years of study by Anahosur and Patil (1981) who suggested that screening of sorghum genotypes against charcoal rot is done better under rainfed condition during *rabi* than *kharif*.

The resistance to this disease as indicated by less lodging of plants associated with larger diameter of basal internodes, shorter peduncles, shorter plant height, thicker rind, higher weight of basal stalk and peduncle section (Esechia *et al.,*1977). Plants with longer growth periods exhibiting non-senescence of leaves were reported to lodge less and were less susceptible to *M. phaseolina* (Rosenow *et al.,* 1977). Such genotypes had higher leaf blade chlorophyll content and 26 per cent higher leaf area duration (LAD) than the other lines screened (Duncan, 1977). Resistance was also related to higher stalk breaking angle and moisture content per unit weight of basal internodes by Indira *et al.* (1984). They suggested that the resistance could be achieved in plants with condensed internodes at the base, which retain moisture upto maturity.

2.1 Collection of *M.phaseolina* isolates

Goidanich and Camici (1947) made a collection of isolates of *M. phaseoli* from bean and Lavender in three widely separated parts of Italy. Similarly, Dhingra and Sinclair (1973b) collected nine isolates of *M. phaseoli* from various parts of United States.

Shekhar *et al.* (2006a) obtained seven isolates of *M. phaseolina* incitant of charcoal rot of maize from different agro-ecological zones of India.

2.1.1 Variability studies

Owing to the highly polykaryotic characteristics of the mycelium, *R. bataticola* (*M. phaseolina)* appears to be highly variable in nature. This may atleast, in part, help to explain, the differences in pathogenecity and cultural characters as reported by several workers (Uppal *et al.,* 1936, Dhingra and Sinclair, 1973a; Ghosh and Sen, 1973; Anilkumar and Sastry, 1980; Gupta and Kolte, 1982; Hooda and Grover, 1982; Byadagi and Hegde, 1985; Arca and Yildizn, 1989; Than *et al.,* 1991; Ali and Dennis 1992 and Shekhar *et al.,* 2006a). Dhingra and Sinclair (1973a, 1973b) noticed variation in growth rate among the isolates from the same plant and between isolates from different plants on different media. Byadagi and Hegde (1985) grouped isolates of bean, *Cicer arietinum* and cowpea as virulent, sorghum and soybean isolates as intermediate and glyricidia isolate as mild, based on, morphology, cultural characters and pathogenic behaviour.

Arca and Yildizn (1989) reported that, out of 362 isolates of Aegean region, 333 exhibited variation in cultural characteristics, such as colony colour, mycelial growth, colony radius and the number of sclerotia. Groups of isolates were established based on radius and the number of sclerotia.

Than *et al.* (1991), conducted colony compatibility tests on 37 isolates of chickpea along with two isolates from different hosts, collected from different locations in Myanmar and reported the possible occurrence of more than one type of *R. bataticola* isolate in a field and the existence of closely related isolates at different places.

Ali and Dennis (1992) isolated *M. phaseolina* from field peas in South Australia and reported that 21 isolates can be differentiated into 15 pathotypes.

Shekhar *et al.* (2006a) made on the basis of colony colour, divided seven isolates into four groups i.e., grayish white, blackish gray, dark black in centre periphery cremish and cottony white colour.

2.1.2 Variation in morphological characters

Uppal *et al.* (1936), reported variation in growth rate and colony character for two sorghum strains of *M. phaseolina* from two different localities viz., Broach farm and Mohol farm in Maharashtra. Cultures of Broach strain showed concentric rings on PDA, while that of Mohol strain, produced aerial mycelium.

Vasudeva (1937) reported that *R. bataticola* grew equally well on Richard’s agar, cotton root extract and synthetic agar but the sclerotia formation was favoured by the last two media and was poor on Brown’s agar. Thomas (1938) observed that the pycnidia of *M. phaseoli* developed in cultures of several solid media.

Goidanich and Camici (1947) observed similar cultural and morphological characters among the strains of *M. phaseoli* isolated from bean (*Phaseolus vulgaris* Linn.) and Lavender.

Khare *et al.* (1970) reported variability in growth pattern and rate of growth among six isolates from root, stem, pod, leaf and seeds of urid bean and soil, when grown on different media. Further they also observed that the soil isolate had the least amount of growth in almost all media while the pod isolate showed maximum growth.

Dhingra and Sinclair (1973a) noticed variation in growth rate among the isolates from the same plant and between isolates from different soybean plants on different media. The stem isolates produced fluffy growth; root isolates showed partially fluffy growth and all other isolates produced appressed growth. Seed and petiole isolates produced concentric alternating zones of sclerotia and this character was more pronounced for the seed isolate. They also collected nine isolates of *M. phaseoli* from different parts of United States and reported variation in growth rate and colony characters. Contrary to this, Sobti and Sharma (1992) reported no correlation between radial growth and virulence or virulence and pycnidial formation in isolates of *R. bataticola* from groundnut in Rajasthan.

Jain *et al.* (1973) isolated *M. phaseolina* (*R. bataticola*) from urid bean plant parts i.e., from root, stem, leaf, pod and seeds and one isolate from the soil, where urid bean crop was taken. In the study for growth on different media, the isolates differed from each other in the growth pattern either in the diameter of colony or nature of aerial mycelium or the type of mycelial growth.

Waseer *et al.* (1990), isolated *M. phaseolina* from soybean and reported that the pathogen grew best on potato dextrose agar or potato dextrose broth at 35^0C. However, little growth occurred on either media at either 20^0C or 40^0C.

2.1.3 Morphology of sclerotia and time taken for sclerotial initiation

Haigh (1928) classified 27 strains of *M. phaseolina* into three groups A, B and C, where C group included those strains having a mean sclerotial diameter of 120 μm or less, the B group included those with about 200 μm, while A group comprised those, whose sclerotia, can conveniently be measured in millimeters. On the basis of differences in size and number of sclerotia, Hildebrand *et al.* (1945) differentiated two strains of *M. phaseolina* isolated from soybean (the Ontario strain), having mean sclerotial diameter of 90.4 × 75.8 μm on host plant and 99.9 × 89.4 μm in culture and from cotton (the Texas strain) whose, sclerotia in culture averaged 85.4 × 73.0 μm and were produced in greater number.

Goidanich and Camici (1947) recorded sclerotial diameter of 65 to 75 μm on host and 100 to 115 μm in cultures with bean isolate and 115 to 135 μm on culture with broad bean (*Vicia fabia* Linn.) isolate. Bean, egg plant, potato, chilli, tomato, Bengal gram and pumpkin isolates produced smooth and round sclerotia, whereas those of cotton tobacco and sesame isolates were more or less irregular (Reichert and Hellinger, 1947). Similarly, studies with eight isolates (B4, B6, B43, B45, B46, B50, B61 and B68) revealed that the sclerotia of B4 and B43 were smaller, less compact and of irregular shape as compared with the other strains (Anon., 1955).

Sclerotial diameter as recorded by Ghosh and Sen (1973) was 65.6 to 114 μm, 45.2 to 81.6 μm, 98.1 to 119.2 μm, 40.2 to 85.0 μm, 52.6 to 80.1 μm, 110 to 114 μm and 89.1 to 116.3 μm with respect to justica (S1), soybean (S2), bengal gram (S3), jute (S7), kochia (S8), groundnut (S9) and red gram (S11) isolates, respectively.

Dhingra and Sinclair (1973b) observed irregular shaped sclerotia in the root isolates and round to elliptical sclerotia in other isolates involved in their study.

Raut and Bhombe (1976) reported that stem isolate of sorghum produced more sclerotial bodies than that of the leaf isolate on Elliott's medium at 30^0C.

Adam and Stokes (1942) observed that mean diameter of sclerotia varied from 63-165 μm on Dox's agar and 50-18 μm on PDA.

Anilkumar and Sastry (1980) reported that the ability of the isolates to produce sclerotia changed with the growth media. Sunflower and horse gram isolates produced maximum number of sclerotia on Rose Bengal Agar (RBA) and Nutrient Agar (NA) respectively. The brinjal isolate produced least sclerotia on NA and no sclerotia on RBA, while, the sesamum and bean isolates did not develop sclerotia on NA. Further, they observed that the sclerotial initiation was quick in sunflower, on all the media tried, while the brinjal and cowpea isolates took maximum time for sclerotial initiation (ten days).

Smits and Noguera (1988) used SEM and light microscopy and reported that the sclerotial formation began with branching and interwining of adjacent hyphal filaments, an increase in size of associated cells and compaction of the sclerotial mass. They also observed that the external cells of peripheral hyphae collapsed, but internally, sclerotia were uniformly reticulate.

Raut and Ingle (1989) reported, differences in sclerotial size of isolates of *R. bataticola* (*M. phaseolina*) from 15 crops including sorghum, soybean and safflower.

Waseer *et al.* (1990), isolated *M. phaseolina* from soybean and reported that, sclerotial size on PDA differed from that on other media, but, was within the range described for *M. phaseolina*.

Dhar and Sarbhoy (1993) isolated the pod and seed isolate of soybean causing ashy stem blight and reported the smallest known sclerotia in the world (mean 32 × 24 µm).

Lokesha and Benagi (2004) recorded maximum sclerotial production on PDA (123.3/microscopic field of 10x). The ICRISAT isolate recorded maximum sclerotial production (71.2/microscopic field), followed by Bidar isolate (68.68/microscopic field).

According to Shekhar *et al.* (2006a) Hyderabad isolate produced highest number of sclerotia (180.3 sclerotia/9 mm disc and 52.0/microscopic 10 × field) of bigger size (95.7 µm), whereas Coimbatore isolate produced minimum number of sclerotia (169 sclerotia) that too with smaller size (66.9 µm).

Shekhar *et al.* (2006a) observed that on the basis of sclerotial morphology, two groups of isolates could be formed, the one with oblong shape having irregular edges and the other being round with regular edges.

Sub species of *Macrophomina phaseolina* were classified based on differences in micro-sclerotial size, cultural characteristics, chlorate sensitivity, pycnidia formation and pathogenecity (Cloud and Rupe, 1988; Mihail, 1992; Karunanithi *et al.,* 1999; Suriachandraselvan and Seetharam, 2000).

2.1.4 Genetic variability

With the advent of new molecular techniques, the use of molecular markers as a tool in comprehensive genetic analysis of crop plants has become important. Since, the first introduction of RFLP markers in genetic mapping (Botstein *et al.,* 1980), molecular markers have opened a new era for plant genetics and breeding. Presently, an array of genetic markers are available to plant geneticists and breeders, which include morphological isozymes, RFLPs, RAPDs, microsatellites sequence tagged sites and AFLPs.

Several types of DNA markers have been used widely (Mohan *et al.,* 1997, Gupta and Varshney, 2000, Babu *et al.,* 2004) for linkage mapping *viz.,* RFLPs (Botstein *et al.,* 1980), RAPDs (Williams *et al.,* 1990),SSRs or microsatellites (Litt and Luty,1989), AFLPs (Vos *et al.,* 1995) and SNPs in combination with DNA chip technology (Wang *et al.,* 1998, Kanazin *et al.,* 2002).

Random amplified polymorphic DNA (RAPD)

It involves the use of single short random oligonucleotide sequence (called random primers) defined cyclic amplification of DNA, which expose the polymorphism, distributed throughout the genome. The amplified fragments one called random amilified polymorphic DNA's (Williams *et al.,* 1990).

Molecular identification of fungus

The fungus is considered to belong to coelomycetes, the presence of teleomorph is yet to be conformed (Mihail, 1992). Subdivisions of this monospecies genus are often based on virulence to particular set of differential host cultivars that vary in disease resistance. Although useful in phytopathology, these tests are subjective (Singh *et al.,* 1990; Wrather *et al.,* 1998; Kendig *et al.,* 2000 and Mayek *et al.,* 1997).

Another important problem concerning *Macrophomina* disease control is non-availability of suitable disease resistant sources for use in breeding (Diourte *et al.,* 1995; Mayek *et al.,* 1997; Smith and Carvil, 1997; Lambhate *et al.,* 2002). Conventional methods are impaired by subjectivity in evaluation of virulence by the inability to characterize non-pathogenic isolates of the fungus and by existence of physiological races (Echavez- Badel and Permodo, 1991). Sub species of *M. phaseolina* were classified based on differences in microsclerotial size, cultural characteristics, chlorate sensitivity, pycnidia formation and pathogenecity (Cloud and Rupe, 1988; Mihail, 1992; Karunanithi *et al.,* 1999 and Suriachandraselvan and Seetharam, 2000).

Su *et al.* (2001) investigated host specialization in *M. phaseolina*. The fungus was isolated from soybean, corn, sorghum and cotton root tissue and soil from fields cropped continuously to these species for 15 years in St. Joseph, L. A. These isolates were examined by RFLP and RAPD analysis. No variations were observed among isolates in restriction pattern of DNA fragments amplified by PCR, covering internal transcribed spacer (ITS) region 5.8 rRNA and part of 25S RNA but variations were observed when ten random (RAPD) primers were used to amplify the total DNA of isolates.

Tarakanta *et al.* (2003) showed that single RAPD primer A13 could be used to identify and discriminate several isolates of *M. phaseolina* and *Fusarium* sp. obtained from 20 hosts including soybean, cotton, chickpea and safflower.

Rajkumar (2004) observed that ten isolates of *M. phaseolina* representing Dharwad and Bijapur regions proved pathogenic to sorghum; molecular profiling using RAPD markers indicated the genetic differences among isolates and were species specific finger print to *M. phaseolina* was identified. Isolates were grouped using molecular and virulence data.

Shekhar *et al. (*2006b), analysed seven isolates of *M. phaseolina* incitant of maize charcoal rot through RAPD marker for genetic diversity. The UPGMA cluster analysis for 706 loci score permitted identification of three main clusters. Similarity matrix and Jaccard's similarity co-efficient between the isolates indicated that the maximum genetic variation was among isolates of Arabhavi and Coimbatore with 70.8 per cent followed by Ludhiana and Coimbatore with 69.5 per cent. The most closely related isolates were Hyderabad and Delhi with an affinity percentage of 65.5 followed by Udaipur and Bangalore isolates with 62.9 per cent similarity.

2.2 Infection of *M. phaseolina*

Karunakar *et al. (*1992) collected the root samples of sorghum seedlings inoculated with *M. phaseolina* at intervals up to 21d, fixed in FAA and examined microscopically. Nectrotic patches were observed on roots 42 h after inoculation. Hyphae penetrated directly and within 24h extended inter- and intracellularly into the epidermal, cortical and vascular tissues. Disintegration of xylem parenchyma, phloem and cortical cells was observed within 3 day. Sclerotia were formed within the host tissues after colonization. Most of the cortical cells were necrotic by the 6^{th} day due to infection whereas, hyphae could not be distinguished. By the 8^{th} day sloughing- off of necrotic tissues from the cortex was noted.

Sinha and Khare (1977) reported that the infection of *M. phaseolina* was observed to be intraembryonal. The mycelium of the pathogen was present in cotyledons, plumule and radicle. The formation of pycnidia of *M. phaseolina* was initiated below the seed coat, on the cotyledons, radicle and plumule. Pycnidia emerged after breaking the seed coat. The embryonal tissues were distorted. Seeds with severe infection rotted completely and the pathogen finally grew on the seed surface.

Basu chaudhary and Pal (1982) observed that the infection of sunnhemp seeds by *M. phaseolina* was either in the form of sclerotia, pycnidia or in mycelium. The pathogen was detected in all the parts of the seed both by component plating and by histopathology. Seeds infected with either sclerotia or pycnidia failed to germinate.

2.3 Screening of genotypes for resistance

Attempts to find out sources of resistance to charcoal rot for breeding programmes were started in USA in the 1940s. In one of the most comprehensive testing programs, Hoffmaster and Tullis (1944) screened 232 sorghum lines of diverse genetic background at four locations for four years. Although, they found differences in the stability in the performance of the lines from year to year, they concluded that it was impossible from the data available to recommend certain varieties for localities in which *Macrophomina* dry rot is a limiting factor. Lack of stability to different levels of drought stress and hence different levels of pre-disposition to the disease was evident.

There have been concerted efforts in evaluation of sorghum germplasm for the disease. Improved cultivars such as CSH 1, CSH 4, CSH 5 and Swarna were found to be susceptible to charcoal rot (Parameshwarappa *et al.,* 1976). Similarly, genotypes *viz.,* SPH 10, SPH 24, SPH 29, CVS 3, SB 1066 and SPV 81 were rated as susceptible (Anahosur and

Rao, 1977). The improved *rabi* variety SPV 86 and hybrid CSH 8R were also reported to be highly susceptible (Avadhani and Ramesh, 1979 and Anahosur *et al.,* 1974). Some low level of resistance was noticed in cultivars like IG 8, CS 3541 (Parameshwarappa *et al.,* 1976) and in a hybrid 36A × 148 (Umate *et al.,* 1975). The incidence of charcoal rot in the B lines was reported to be greater than A lines (Patil and Thombre, 1979).

Contrary to this, local varieties like M35-1, 5-4-1 and A1 were highly tolerant (Avadhani and Ramesh, 1979; Anahosur *et al.,* 1978; Gowda *et al*., 1981). However E36-1, an Ethiopian accession, has consistently shown resistance to lodging at several locations in three years of testing. The plants of this genotype were infected by the fungus (roots and stalks), but the infection was not severe enough to cause lodging (Hoffmaster and Tullis, 1944; Anahosur and Patil, 1982, 1983).

In a study at ICRISAT, 55 direct hybrids and their 11 parental lines were evaluated for charcoal rot resistance by measuring lodging, stalk infection and susceptibility index. All the three parameters were reported to be governed by high degree of dominance and low heritability. In a study of diallel crosses involving four resistant and three susceptible varieties for charcoal rot, Garud and Borikar (1985) observed predominance of dominance for resistance with over-dominance and low heritability values for number of nodes crossed and length of stem infected by the pathogen.

Resistance to *M. phaseolina* in each environment and across environments was found to be related to maturity and the stalk disintegration scores were significantly negatively correlated both phenotypically and genotypically (Bramel-Cox, 1995).

2.3.1 Screening techniques for resistance

2.3.1.1 Artificial inoculation techniques

The procedure followed by most investigators is essentially that of Edmunds *et al.* (1964).Sorghum is grown under rainfed condition, in an environment known to be favourable for charcoal rot. Drought stress is induced by with holding irrigation at selected stages of plant maturity. The stalks are inoculated by inserting tooth-picks loaded with mycelia and sclerotia into holes made just above the first node. Amount of lodging, soft stalks and the spread of the fungus from the point of inoculation on the stem are the three measurements taken in assessing reaction of the genotypes to the disease.

The stem tape inoculation technique developed by Mayee and Garud (1978) involves multiplication of the causal organism on sorghum grain media and placing these inoculum loaded grains directly on non-injured first or second internode from ground level in 45 day old sorghum plants and covering with a cellophane tape.

At ICRISAT, Mughogho and Pande (1984) induced charcoal rot without artificial inoculation in field susceptible grain sorghum by two methods. In the first method crop is sown just before the end of the rainy season so that it grows and matures under progressively less soil moisture. This is to ensure conditions similar to that of the post-rainy season (*rabi*) crop in India, in which usually crop suffers from charcoal rot. The other method is to grow the crop under irrigation during the dry season and withdraw irrigation at 50 per cent flowering. Disease development in susceptible genotypes was sufficiently high for useful evaluation of test genotypes.

Toothpick inoculation and other methods where inoculum is introduced into the plant through the stalk are unsatisfactory, primarily, because they do not closely simulate the natural infection process, which begins in the roots and later spreads to the stem. Furthermore, the level of disease development with toothpick inoculum is usually less than that which occurs naturally and is therefore unsatisfactorily for assessing resistance that could be useful under natural conditions (Edmunds *et al.,* 1964).

3. MATERIAL AND METHODS

The present investigation was carried out during 2005-07 at Department of Plant Pathology, Main Agricultural Research Station (MARS) and the Institute of Agri-Biotechnology (IABT), University of Agricultural Sciences, Dharwad. The details of material used and the methodologies adopted are described in this chapter.

General procedure

Glassware and cleaning

Corning glasswares were used for all the experiments carried out. The glasswares were kept in the cleaning solution for a day containing 60 g of potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated H_2SO_4 in one litre of water. They were washed with detergent powder followed by washing in running tap water and rinsing with distilled water couple of times.

Sterilization

All glasswares were dried in a hot air oven at 160°C for two hrs. Both solid and liquid culture media were sterilized at 1.1 kg pressure per sq. cm for 20 min.

3.1 Study material

3.1.1 Collection of diseased specimens

Charcoal rot affected sorghum plants were collected from different parts of Karnataka (Dharwad, Gadag, Athani, Raichur, Gulberga, Bidar, Bijapur), Maharashtra (Zalki, Koparagoan, Pune, Shirdi, Rahuri, Ahmednagar, Parbhani, Mulegoan, Solapur, Tulajapur, Ushmanabad, Amaravati, Jalna, Nanded, Akola) and Andhra Pradesh (Ramnagar, Patancheru and Nizamabad) constituting 26 infected sorghum samples in all.

All 26 diseased samples were collected for the purpose of variability study. Details of location and designation given for each isolate are furnished in Table 1.

3.1.2 Isolation, identification and maintenance of *M. phaseolina*

In all the samples infected stalk was used for isolation. Infected portion of stalk was cut into small pieces of 5 to 7 mm and the bits were surface sterilized with 1:1000 (0.1%) mercuric chloride solution for one min. followed by three times wash with sterile water and then transferred to petriplates containing sterilized potato dextrose agar (PDA). These plates were incubated at 27±1°C for four days to obtain good growth of fungi. The composition of the media used is furnished below.

Potato Dextrose Agar (Tuite, 1969)

Peeled and sliced potatoes	200 g
Dextrose ($C_6H_{12}O_6$)	20 g
Agar-agar	20 g
Distilled water (to makeup)	1000 ml

The potatoes were boiled in 400 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in 400 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was added to the mixture. The volume was made up to 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

The principle growth characters like morphological, cultural and formation of sclerotia were considered for identification of pure cultures of *M. phaseolina*. These characters were compared as described by Ashby (1927) and Goidanich (1947) and identified as *M. phaseolina* depending on the observed traits. Subsequent subculturing was done once in 15 days on PDA slants and was preserved in refrigerator at 4°C for further experiments.

Table 1: Location and plant part chosen for isolation purpose

Sl. No.	Location	Isolate designation	Plant part chosen for isolation
A.	**Karnataka**		
1.	Dharwad	Dh	Infected stalk
2.	Gadag	Ga	Infected stalk
3.	Athani	At	Infected stalk
4.	Raichur	Ri	Infected stalk
5.	Gulberga	Gu-1 Gu-2	Infected stalk Infected stalk
6.	Bidar	Bo	Infected stalk
7.	Bijapur	Bj	Infected stalk
B.	**Maharashtra**		
1.	Zalki	Za	Infected stalk
2.	Kopargoan	Ko	Infected stalk
3.	Pune	Pu	Infected stalk
4.	Shirdi	Sh	Infected stalk
5.	Rahuri	Ra	Infected stalk
6.	Ahmednagar	Ah	Infected stalk
7.	Parbhani	Pr	Infected stalk
8.	Mulegoan	Mu	Infected stalk
9.	Solapur	So	Infected stalk
10.	Tulajapur	Tu	Infected stalk
11.	Ushmanabad	Us	Infected stalk
12.	Amaravati	Am	Infected stalk
13.	Jalna	Ja	Infected stalk
14.	Nanded	Na	Infected stalk
15.	Akola	Ak	Infected stalk
C.	**Andhra Pradesh**		
1.	Ramnagar	Rm	Infected stalk
2.	Patancheru (ICRISAT)	Pa	Infected stalk
3.	Nizamabad	Nz	Infected stalk

3.1.3 Studies on variability among the isolates

The possibility of existence of variability among 26 isolates collected from different locations of Karnataka, Maharashtra and Andhra Pradesh were attempted through a series of experiments.

3.1.4 Morphological characters on PDA

About 15 ml of the medium was poured into each of the 90 mm diameter Petri dishes. One millilitre of streptomycin sulphate solution of 100 ppm strength was added to the medium just before pouring into the plates to avoid bacterial contamination. Inoculation was made by transferring half a centimeter disc of mycelial mat, taken from the periphery of seven day old culture of each of the 26 isolates. The plates were incubated at 27 ± 1°C.

Differences in topography, colour of colony, rate of growth, size, shape and number of sclerotia (on PDA) were recorded.

For each of the 26 isolates grown on PDA, diameter of sclerotial bodies were recorded with the help of standardized filar micrometer and converted into microns. The range and mean of sclerotial size was calculated and statistically analysed.

3.1.5 Cultural characters on potato dextrose agar

Colony diameter on PDA media (Tuite, 1969) was recorded from the second day, till the growth completely covered the plates.

3.1.6 Molecular characterization of isolates of *Macrophomina phaseolina*

Random amplified polymorphic DNA (RAPD) analysis was used to detect the variation among the isolates of *M. phaseolina..* Standard protocol as described by Rajkumar (2004) was followed to isolate the DNA and RAPD analysis.

Requirements

Stock solutions

1.2% SDS

2. Lysis buffer

    2.5 mm EDTA

    1% Triton x

    50 mM Tris-HCl

3. Phenol:Chloroform(1:1)

4. 3M sodium acetate

5. Isopropanol

6. T10E1

    10 mM tris HCl

    1 mM EDTA, pH 8.0

7. 100 □M random primers

8. 25 ng □l^{-1} Template DNA

9. 3-0 U □l Taq DNA polymerase

3.1.7 Isolation of genomic DNA

The genomic DNA was isolated from fungi following the protocol of Rajkumar (2004).

Three grams of fungal mycelial mat grown on potato dextrose agar was taken and homogenized using pestle and mortar in 4 ml of 2 per cent sodium dodecyl sulfate (SDS) for 5 min. To the above solution, 6 ml of lysis buffer (2.5 Mm EDTA, 1% Triton x and 50 mM Tris-HCl, pH 8.0) was added. The suspension was extracted with equal volumes of phenol: chloroform (1:1) and centrifuged at 10,000 rpm for 10 min. The supernatant was taken into a fresh tube and $1/10^{th}$ volume of 3 M sodium acetate and 0.54 volume of isopropanol were added at room temperature, mixed by gentle inversion and kept for 30 min at 2°C. The DNA was recovered by centrifugation at 10,000 rpm for 10 min. at 4℃. The DNA pellet was washed with 70 per cent ethanol, air-dried and re-suspended in 300 µl of T10E1 (10 mM tris HCl and 1 mM – EDTA, pH 8.0).

Purification of genomic DNA

The genomic DNA isolated was purified according to the protocol described by Rajkumar (2004).

To the DNA solution, RNase @ 100 µg per ml was added and this solution was incubated for two hours at 37℃ in a water bath. The solution was centrifuged at 10,000 rpm for 10 min. and the suspension was treated with equal volume of buffered phenol (pH 8.0) and centrifuged. The upper aqueous layer was taken in a fresh tube and treated with equal volume of phenol: chloroform (1:1 v/v). This suspension was centrifuged and upper aqueous layer was taken into fresh tube and to this 1/10th volume of 3M sodium acetate and 2 volumes of absolute ethanol were added and incubated at 4℃ for 2 hrs. The DNA was pelleted by centrifugation at 10,000 rpm for 10 min. The pellet was washed with 70 per cent ethanol, air dried and dissolved in 100 □l of T10E1 buffer and stored at 4℃ until further use.

3.1.8 Molecular characterization of *M. phaseolina*

RAPD was carried out for molecular characterization of the isolates as per the protocol described by Rajkumar (2004).

Primers for RAPD

Random primers (10 pM) were used for molecular characterization of 26 isolates of *M. phaseolina*. Commercial kit OPA series of decamer DNA primers were obtained from M/s Integrated DNA tecnologies supplied by Sigma Industrial and laboratory Equipments Inc.,Bangalore, India.

dNTP's

The four individual dNTP' s such as dATP, dGTP, dCTP and dTTP were obtained from M/s Bangalore Genei, Pvt. Ltd.,Bangalore.

Taq DNA polymerase

Taq DNA polymerase and 10 x Taq buffer were obtained from M/s Bangalore Genei, Pvt, Ltd., Bangalore.

Thermo cycler

Corbett Research gradient PCR supplied by M/ s. JH Bio Innovation Pvt. Ltd., R.T. Nagar Bangalore was used for cyclic amplification of DNA.

Reaction mixture:

Amplification reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing following components .The total volume of each reaction mixture was 25 µl. The following reaction mixture was found to be optimum for PCR amplification.

Sl. No.	Components	Quantity (µl/reaction)
1.	Template DNA	2.0
2.	dNTPs mix (2.5 mM each)	1.0
3.	Primer (10 pM/ml)	1.0
4.	$MgCl_2$(25 µM)	1.0
5.	10 x Assay buffer	2.5
6.	Taq DNA polymerase	0.34
7.	Sterile distilled water	17.16

Except template, the master mix was distributed to PCR tubes (23 µl / tube) and later 2 µl of template DNA from the respective isolates were added making the final volume of 25 µl.

Polymerase chain reaction (PCR) conditions for RAPD analysis

The PCR amplification for RAPD analysis was performed according to Rajkumar (2004).The optimum conditions for DNA amplifications used were as follows.

Stage	Step	Temperature (°C)	Duration (min)	No. of cycles
I.	Initial denaturation	94	10	1
II.	Denaturation	94	1	39
	Annealing	36	1.2	
	Extension	72	2	
III.	Final extension	72	10	1

After the completion of the PCR, the products were stored at 4 ºC until the gel electrophoresis was done.

A total of 20 random primers with the following sequences were used in the study.

Sequences of random primers used in RAPD analysis for different isolates of *M. phaseolina*

Primer	Sequence 5^{1} - 3^{1}
OPA-01	CAG GCC CTT C
OPA-02	TGC CGA GCT G
OPA-03	AGT CAG CCA C
OPA-04	AAT CGG GCT G
OPA-05	AGG GGT CTT G
OPA-06	GGT CCC TGA C
OPA-07	GAA ACG GGT G
OPA-08	GTG ACG TAG G
OPA-09	GGG TAA CGC C
OPA-10	GTG ATC GCA G
OPA-11	CAA TCG CCG T
OPA-12	TCG GCG ATA G
OPA-13	TTG ATC GCA C
OPA-14	TCT GTG CTG G
OPA-15	TTC CGA ACC C

OPA-16	AGC CAG CGA A
OPA-17	GAC CGC TTG T
OPA-18	AGG TGA CCG T
OPA-19	CAA ACG TCG G
OPA-20	GTT GCG ATC C

Separation of amplified products by agarose gel electrophoresis

Requirements

1. Electrophoresis unit: Gel casting trough, gel combs, power pack, UV Transilluminator.
2. Agarose (1.2 %)
3. Bromophenol blue
4. Ethidium bromide (0.5 µg ml^{-1})
5. 50 x TAE (stock): tris-base-60.5 g

   Glacial acetic acid – 14.25 ml

   0.5 M EDTA – 25 ml

   Make up the volume to 250 ml, pH 8.0
6. Working solution (1 x TAE): 20 ml of 50 x TAE was made upto 1000 ml by using distilled water.

Procedure

- Three grams of agarose was weighed and added to a conical flask containing 250 ml of 1 x TAE buffer.
- The agarose was melted by heating the solution in an oven and the solution was stirred to ensure even mixing and complete dissolution of agarose.
- The solution was then cooled to about 40-45ºC.
- Two to three drops of ethidium bromide (0.5 µg ml^{-1}) was added.
- The solution was mixed and poured into the gel casting platform after inserting the comb in the trough. While pouring sufficient care was taken for not allowing the air bubbles to trap in the gel.
- The gel was allowed to solidify and the comb was removed after placing the solidified gel into the electrophoretic apparatus containing sufficient buffer (1 x TAE) so as to cover the wells completely.
- The amplified products (20 µl) to be analysed were carefully loaded into the sample wells, after adding bromophenol blue with the help of micropipette.
- Electrophoresis was carried out at 60 volts, until the tracking dye migrated to the end of the gel.
- Ethidium bromide stained DNA bands were viewed under UV transilluminator and photographed for documentation.

Scoring the amplified fragments

The amplified products along with 2 µl of loading dye (Bromophenol blue) were separated on 1.2 per cent agarose gel at 80 volts (45 volts per cm of gel) using 1 x TAE buffer of pH 8.0 containing ethidium bromide (0.5 µl/10 ml of gel). The amplified profiles for all the primers were compared with each other and the bands of DNA fragments were scored as 1 for the presence and 0 for the absence of a band generating the '0', '1' matrices. The gels were photographed using gel documentation system.

Statistical analysis

DNA bands that could be scored unequivocally for presence or absence were included in the analysis. Faint bands were not scored. Binary matrices were analyzed by NTSYS-PC (version 2.0; Exeter Biological Software, Setauket, NY). Jacard's coefficients were clustered to generate dendrogram using the SAHN clustering programme, selecting the unweighted pair group method with arithmetic average (UPGMA) algorithm in NTSYS-PC (Rohlf, 1998), using the above programme other parameters computed were

$$\text{Per cent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

3.2 Studies on mode of infection of *M. phaseolina* in sorghum roots

- Seeds of charcoal rot susceptible (SPV – 86) and resistant (E36 – 1) cultivars were soaked in sterilized distilled water for 3-4 hrs.
- Individual seeds were then placed in moist blotter sheets and allowed the seeds to germinate.
- After emergence, roots of the seedlings were dipped in the inoculum (virulent isolate D_5 obtained from the Institute of Agri-Biotechnology (IABT), Dharwad) and kept for some time.
- The root samples were collected at an interval of 24 hr, 48 hr, 3rd, 6th, 8th and 10th day after dipping in the inoculum.
- The infection and further development of mycelium inside the roots were observed by staining the roots using lacto phenol and cotton blue dye and the photographs were taken by using Motic Images 2.0 software.

3.3 Evaluation of RILs

3.3.1 Experimental materials

The experimental materials used consisted of a set of 93 recombinant inbreed lines (RILs) and two contrasting parents for resistance (E36-1) and susceptible(SPV 86) to charcoal rot disease of sorghum. The mapping population was obtained from Institute of Agri-Biotechnology (IABT), UAS, Dharwad. The two parents were IS22380, a Sudanean dwarf line (susceptible to charcoal rot) and E36-1 a stay green source adapted to tropics (resistant to charcoal rot).

Experiment was carried out at Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad during *rabi* 2006-07.

3.3.2 Soil sterilization

- Soil free from clumps and stones was obtained and mixed thoroughly with compost in 6:1 proportion.
- The cleaned soil was transferred to cement tank (pot) and 4% formalin was added to every layer of soil in order to sterilize the soil.
- The soil surface was made air tight by covering with polythene sheet.
- The polythene sheet was removed after 48 hours and soil was spread to facilitate escape of toxic fumes, if any.

Completely Randomized Design was adopted and seeds were sown in pots (9” × 9”) in two replications.

3.3.3 Screening for resistance

3.3.3.1 Tooth pick method

M. phaseolina was cultured on tooth picks using honey peptone medium as described by Rao *et al.* (1978). Honey peptone medium was prepared by dissolving one g of peptone in 5 ml of honey and 94 ml of distilled water.

Tooth picks were boiled in water for two hours to remove toxic substances that may inhibit the growth of fungi. After boiling, they were washed thoroughly in fresh tap water, when tooth picks became dry, about 10 tooth picks were placed in 100 ml flasks, in such a way that the, pointed end of tooth picks faced away from the base and was sterilized at 1.1 kg/cm^2 for 20 min.

The honey peptone broth was sterilized at 1.1 kg/cm^2 for 15 min in sixty 100 ml flasks. After cooling, *M. phaseolina* isolate (D5) was inoculated to two 100 ml flasks and a rich suspension was made. This suspension was poured into two tooth pick flasks to cover lower one third of the tooth picks.

The flasks were incubated for seven days at 30°C, by the time, tooth picks were covered with the fungal growth and were ready for inoculation. A pointed iron needle (1-2 mm diameter) with a wooden handle was used to make a hole position in the stem, to facilitate tooth pick insertion. Tooth picks were introduced obliquely into the sorghum stalk at the second internode 15 days after flowering. Care was taken to ensure that drought stress conditions prevailed at the time of toothpick insertion.

Three parameters *viz*., per cent lodging, number of internodes crossed and length of spread by the fungus were used for assessing the reaction of the genotypes to the pathogen. Disease incidence was recorded 20 days after complete grain filling. Further observations were also recorded on number of nodes crossed and length of spread (cm) was measured from the lowest node of the stem.

4. EXPERIMENTAL RESULTS

The present investigation on morphological and molecular variability and host resistance response of sorghum recombinant inbreed lines (RILs) to a virulent isolate of *Macrophomina phaseolina* (Tassi) Goid. was conducted at Department of Plant Pathology, Main Agricultural Research Station (MARS) and the Institute of Agri-Biotechnology (IABT), College of Agriculture, University of Agricultural Sciences, Dharwad during 2005-07. The results of the experiments are presented hereunder.

4.1 Collection of diseased samples and isolation of the fungus

Twenty six charcoal rot infected sorghum samples were collected from different parts of Karnataka (Dharwad, Gadag, Athani, Raichur, Gulberga, Bidar and Bijapur), Maharashtra (Zalki, Kopargoan, Pune, Shirdi, Rahuri, Ahmednagar, Parbhani, Mulegoan, Solapur, Tulajapur, Ushmanabad, Amaravati, Jalna, Nanded, Akola) and Andhra Pradesh (Ramnagar, Patancheru and Nizamabad). In all the samples infected stalk was used for isolation.

Isolation of the fungus was done following standard isolation procedure as described in material and methods.

4.1.1. Identification of the fungus

The growth of fungal mycelium on Potato Dextrose Agar (PDA) was fast. Initially, it was hyaline and turns brown to black as it grew old. Abundant sclerotial production was observed. The sclerotia were spherical to irregular, dark brown, varying in size and were connected to mycelium. Hyphae were branched and sparsely septate. Pycnidia were not observed in all the cultures.

The morphological characters observed were almost similar to those described by Ashby (1927) and Goidanich (1947) and hence the fungus under study was identified as *M. phaseolina.*

4.1.2 Studies on variability in *Macrophomina phaseolina*

4.1.2.1 Morphological variability

Morphological features of 26 isolates of *M. phaseolina* were observed by growing the isolates on PDA medium. Different parameters like colony characters, sclerotial size, days taken for sclerotial formation, and number of sclerotia per microscopic field were considered to assess the possible variation among isolates.

Based on colony colour, the cultures were assigned to four groups *viz.,* grayish white (Dh, Us, Pr); blackish gray (Ri, Ga, Gu-1, Gu-2, Bo, Rm, Za, So, Pu, Ah, Ko, Na, Ja, Nz, Ak); deep black (Bj, At, Am, Mu, Tu, Ra); cottony white colour (Pa and Sh). Isolates could also be assigned into two groups, on the basis of colony texture. Dh, Am, Sh, Pr isolates produced fluffy colony, while Ri, Bj, Ga, Gu-1, Gu-2, Bo, At, Rm, Za, Mu, So, Tu, Us, Pu, Ah, Ko, Ra, Na, Ja, Nz, Ak isolates produced flat colony. Mean colony diameter of 26 isolates differed significantly four days after inoculation on PDA (Table 2).

Based on mean colony diameter the isolates grouped into four categories (Table 3). The first group consisted of 18 isolates with a spread of 80.37 to 90.00 mm, second group included 4 isolates (Gu-1, Bo, Pr and Ja) with a mean growth of 70.73 to 80.36 mm, third group included only one isolate Ra with a mean growth of 67.00 mm. While, the fourth group included 3 isolates (Bj, Za and Tu) with a mean growth of 51.45 to 61.08 mm indicating, the existence of variation among the isolates (Fig. 1).

The size of sclerotia varied from 94.0 µm to 30.0 µm (Table 4). It was observed that the Rm isolate produced largest sclerotia of 94.0 µm, while the smallest size sclerotia of 30.0 µm was produced by Bijapur isolate. Round shaped sclerotia were observed in Ri, Bj, Ga, Gu-1, Gu-2, At, Pa, Za, Mu, Tu, Pu, Ah, Ko, Sh, Na, Ja, Nz and Ak isolates, while, the oblong shape of sclerotia were observed in Dh, Bo, Rm, Am, So, Us, Ra and Pr isolates.

Based on average sclerotial size, the isolates were categorized into eight groups (Table 5); six isolates (Rm, Am, Za, So, Us and Ak) recorded maximum size ranging from 87.65 µm to 96.10 µm followed by the second group, which included three isolates, (Pu, Ah and Ko) with a sclerotial size of 79.17 µm to 87.64 µm, fourth group included only one isolate, Pa with a sclerotial size of 70.6 µm, fifth group included five isolates Bo, At, Mu, Tu and Ja with a sclerotial size ranging from 53.73 µm to 62.20 µm, sixth group included three isolates Ri, Pr and Na with a sclerotial size ranging from 45.25 µm to 53.72 µm. seventh group included only two isolates Gu-1 and Nz with a sclerotial size of 36.77 µm to 45.24 µm, while, the last group included six isolates (Bj, Ga, Gu-2, Dh, Ra and Sh) recording the lowest sclerotial size ranging from 28.29 µm to 36.76 µm.

Most of the isolates took two to three days for sclerotial formation, except Bj isolate, which took four days for sclerotial formation. Maximum number of sclerotia of 60.3 per microscopic field 10x was recorded in Ra isolate, while the minimum number of 39.6 was observed in So isolate (Table 6).

The number of sclerotia/9 mm disc varied from 110-50 during second day, 135-72 during fourth and 180-100 during sixth day after inoculation (Table 7). Among the isolates Ra isolate had maximum number of sclerotia/9 mm disc (110, 135 and 180) during 2^{nd}, 4^{th} and 6^{th} day after inoculation, while, the minimum number was observed in So (50) during 2^{nd} DAI, Us (72) during 4^{th} DAI and So, Us and Ah isolates (100) during 6^{th} DAI (Fig. 2).

The observations on length and breadth of sclerotia are presented in Table 8. The length and breadth of sclerotia varied from 36.16-85.14 µm $\times$ 21.96 – 62.72 µm was observed that the Ak isolate produced higher length and breadth of 85.14 µm $\times$ 62.72 µm , while the lower length and breadth of 36.16 $\times$ 21.96 µm was noticed in Bo isolate.

4.1.2.2 Genetic variability

Genetic variation was detected among the 26 isolates of *M. phaseolina* using 20 RAPD markers (Table 9). Of the 20 primers used for amplification, OPA1, OPA2, OPA3, OPA4, OPA6, OPA7, OPA9, OPA11, OPA12, OPA13, OPA14, OPA15, OPA17, OPA18, OPA19 and OPA20 showed cent per cent polymorphism among isolates. A total of 189 amplicon levels resulted from 20 primers and were available for analysis. Information on banding pattern for all the primers were used to determine genetic distance between isolates and to construct a dendrogram.

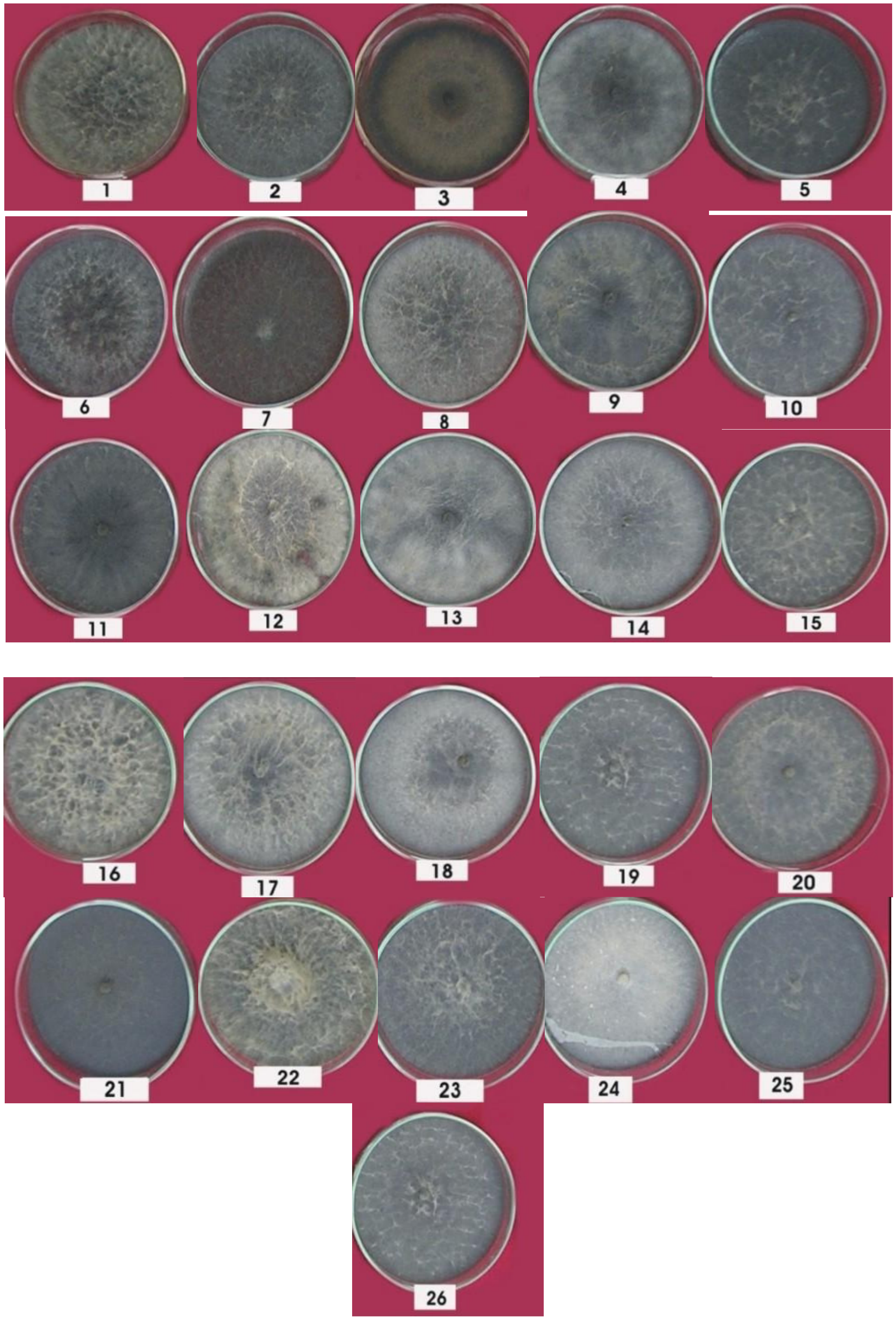
Based on simple matching co-efficient a genetic similarity matrix was constructed to assess the genetic relatedness among the isolates of *M. phaseolina*. Genetic similarity coefficient of 26 isolates of *M. phaseolina* based on RAPD analysis is given in the Table 10. Similarity coefficient ranged from 0.12 to 0.63. The maximum genetic similarity of 63 was between Ja and Pu, where as least genetic similarity was observed between Us and Mu isolates.

The dendrogram for pooled data (of primers) showed five major clusters (Fig. 3). The isolates Mu, Dh, Pu, Ja, Pa, Gu-1, Ko, So and Sh were found in one cluster, isolate Bj in a separate cluster, Rm, Pr, Ri, Am, Ju, At isolates were found in another cluster, Ah, Ra, Ak, Ga isolates were found in one cluster and Nz, Us, Na, Za, Gu-2 and Bo isolates were found in a separate cluster.

4.2 Path of infection

Small discoloured specks were observed on the roots 24 hr after inoculation indicating a possible biochemical effect of the pathogen. After 48 hr, minute necrotic lesions were developed. By the 3^{rd} day necrotic patches in more than one place were observed. On the 6^{th} day, 25 per cent of the area was necrotic and upto 50 per cent of the area was necrotic by the 8^{th} day. On the 10^{th} day, 75 per cent of the root area had become necrotic in both susceptible (SPV-86) and resistant (E36-1) varieties.

Abundant hyphae of *M. phaseolina* were observed on the surface of roots after 24 hrs of inoculation. No hyphae were observed within the root hairs. The sclerotia germinated on the surface of roots 24 hr after inoculation. The mycelium of *M. phaseolina* penetrated the roots directly in 24 hr after inoculation. The root hairs lost their turgidity and began to deteriorate. After penetration, the hyphae extended inter and intracellularly into the epidermal and cortical tissues in both susceptible (SPV-86) and resistant (E36-1) varieties.



1. Amaraavati 2. Raichur 3. Bijapur 4. Zalki 5. Mulegoan 6. Solapur 7.Tulajapur 8. Ushmanabad 9. Pune 10. Ahmednagar 11. Rahuri 12. Shirdi 13. Kopargoan 14. Gadag 15. Gulberga-1 16. Parbhani 17. Nanded 18. Jalna 19. Nizamabad 20. Gulberga-2 21. Athani 22. Dharwad 23. Ramnagar 24. Patancheru 25. Bidar 26. Akola

Plate 1: Photograph showing growth of different isolates on potato dextrose agar (PDA)

Table 2: Growth characters of 26 isolates of *M. phaseolina* from sorghum

Isolates	Mean colony diameter(mm)	Colony colour	Colony appearance
Ri	90.00	Blackish gray	Flat colony
Bj	60.00	Deep black	Flat colony
Ga	90.00	Blackish gray	Flat colony
Gu-1	77.66	Blackish gray	Flat colony
Gu-2	90.00	Blackish gray	Flat colony
Dh	88.00	Grayish white	Fluffy colony
Bo	79.33	Blackish gray	Flat colony
At	88.00	Deep black	Flat colony
Rm	89.66	Blackish gray	Flat colony
Pa	85.00	Cottony white	Flat colony
Am	90.00	Deep black	Fluffy colony
Za	60.33	Blackish gray	Flat colony
Mu	90.00	Deep black	Flat colony
So	90.00	Blackish gray	Flat colony
Tu	60.00	Deep black	Flat colony
Us	88.00	Grayish white	Flat colony
Pu	88.33	Blackish gray	Flat colony
Ah	90.00	Blackish gray	Flat colony
Ko	90.00	Blackish gray	Flat colony
Ra	67.00	Deep black	Flat colony
Sh	90.00	Cottony white	Fluffy colony
Pr	80.33	Grayish white	Fluffy colony
Na	90.00	Blackish gray	Flat colony
Ja	75.00	Blackish gray	Flat colony
Nz	90.00	Blackish gray	Flat colony
Ak	88.33	Blackish gray	Flat colony
SEm $\pm$	0.942		
CD at 1%	3.55		

Table 3: Grouping of 26 isolates of *M. phaseolina* based on colony diameter (mm) on PDA media

Group	Range (mm)	No. of isolates	Isolates
A	90.00-80.37	18	Ri, Ga, Gu-2, Dh, At, Rm, Pa, Am. Mu, So, Us, Pu. Ah, Ko, Sh., Na, Nz, Ak
B	80.36-70.73	4	Gu-1, Bo, Pr, Ja
C	70.72-61.09	1	Ra
D	61.08-51.45	3	Bj, Za, Tu

CD at one per cent is 3.55

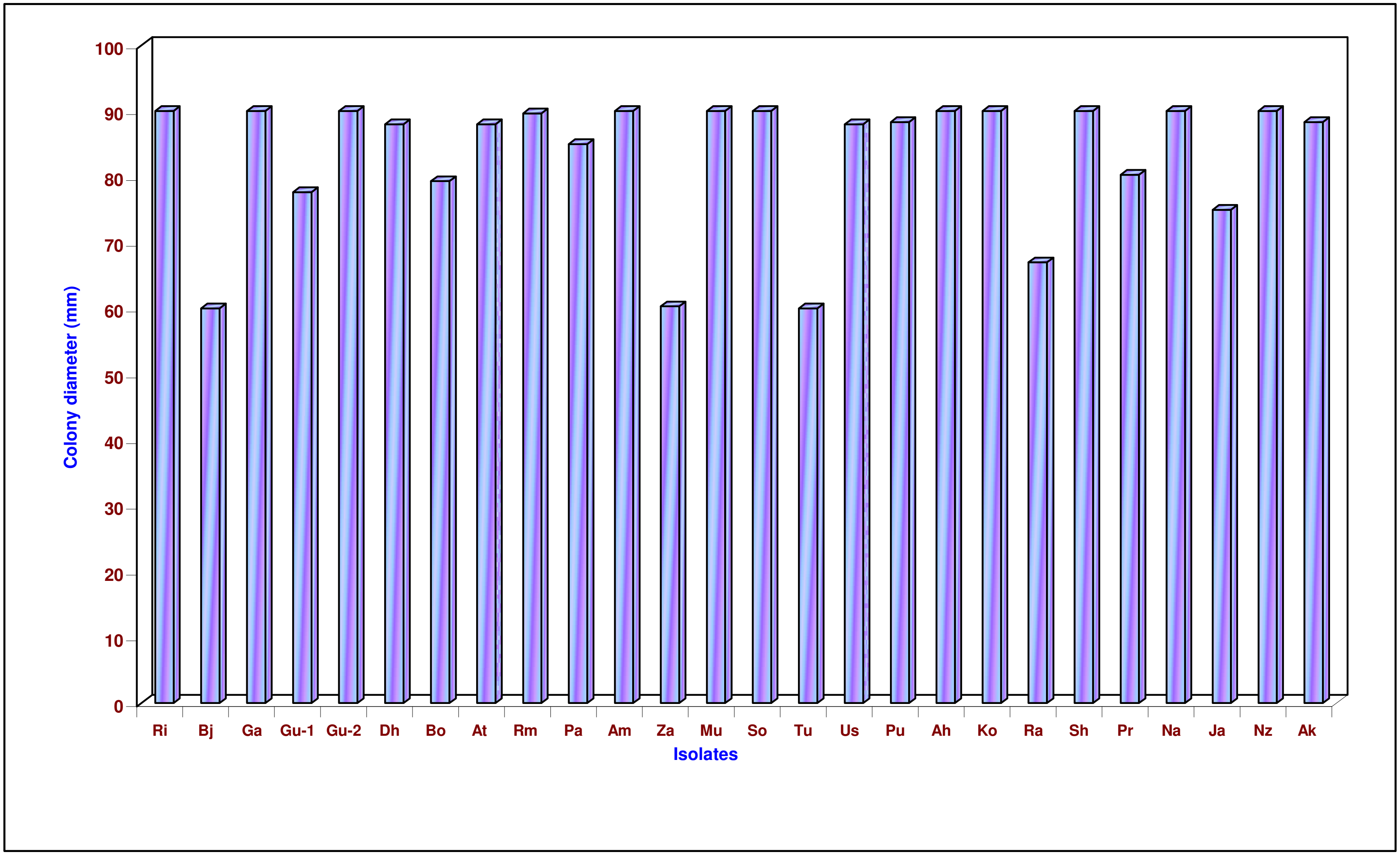


Fig. 1: Colony diameter (mm) of M. phaseolina isolates on potato dextrose agar medium four days after inoculation

Table 4: Size and shape of sclerotia produced by different isolates of *M. phaseolina*

Isolates	**Sclerotial diameter (µm)**	**Shape of sclerotia**
Ri	45.3	Round
Bj	30.0	Round
Ga	33.3	Round
Gu-1	39.3	Round
Gu-2	35.3	Round
Dh	36.0	Oblong
Bo	62.0	Oblong
At	60.6	Round
Rm	94.0	Oblong
Pa	70.6	Round
Am	89.3	Oblong
Za	88.6	Round
Mu	60.6	Round
So	93.3	Oblong
Tu	62.0	Round
Us	88.6	Oblong
Pu	84.6	Round
Ah	84.0	Round
Ko	81.3	Round
Ra	34.0	Oblong
Sh	36.0	Round
Pr	51.3	Oblong
Na	49.3	Round
Ja	58.0	Round
Nz	40.6	Round
Ak	88.0	Round
SE m±	0.716	-
CD at 1%	2.70	-

Table 5: Grouping of isolates based on average sclerotial size (□m)

Group	Range of sclerotial size	Number of isolates	Isolates
1	96.10-87.65	6	Rm, Am, Za, So, Us, Ak
2	87.64-79.17	3	Pu, Ah, Ko
3	79.16-70.69	-	-
4	70.68-62.21	1	Pa
5	62.20-53.73	5	Bo, At, Mu, Tu, Ja
6	53.72-45.25	3	Ri, Pr, Na
7	45.24-36.77	2	Gu-1, Nz
8	36.76-28.29	6	Bj, Ga, Gu-2, Dh, Ra, Sh

CD at one per cent is 2.70

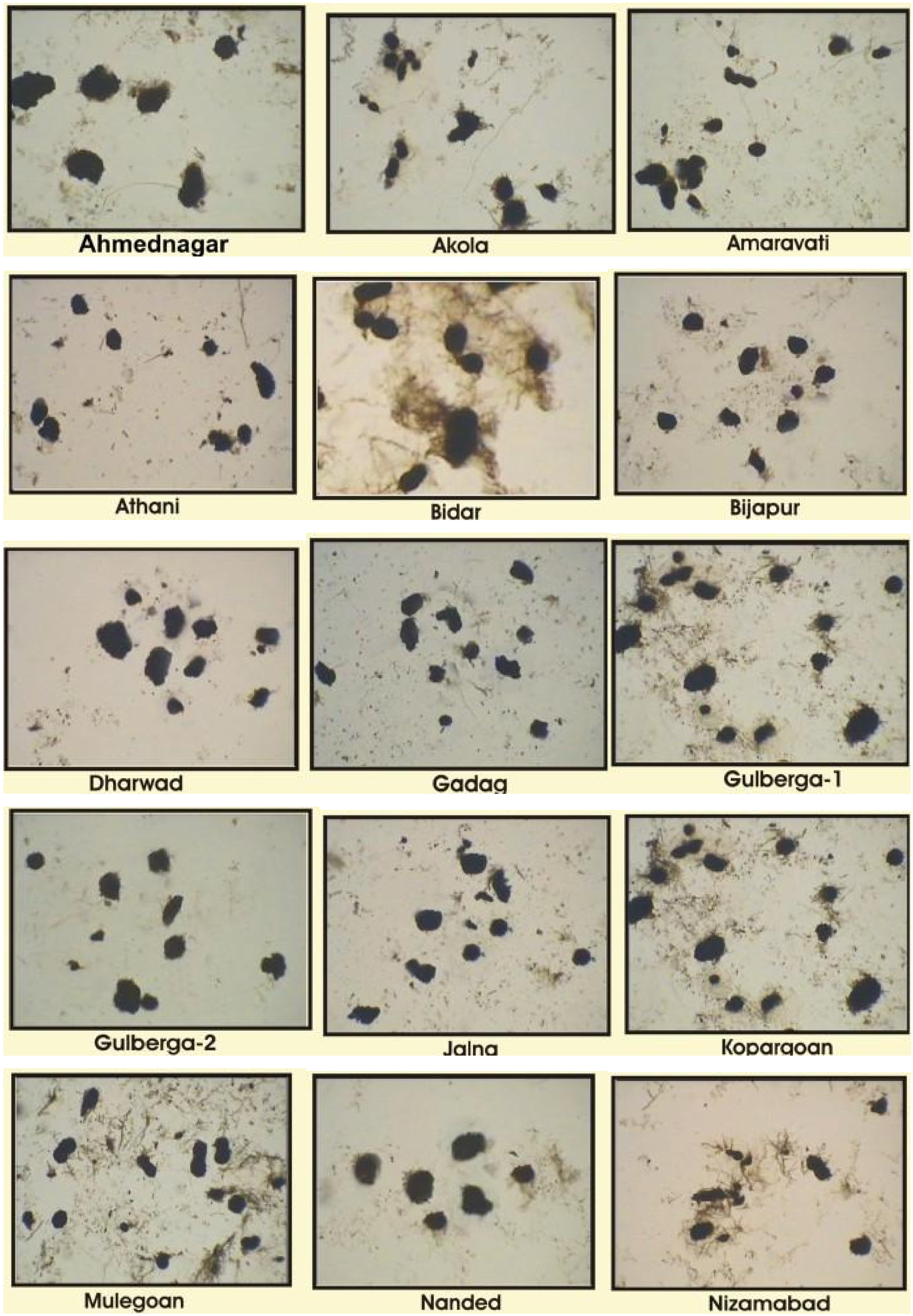


Plate 2: Sclerotial morphology and shape of different isolates of M. phaseolina

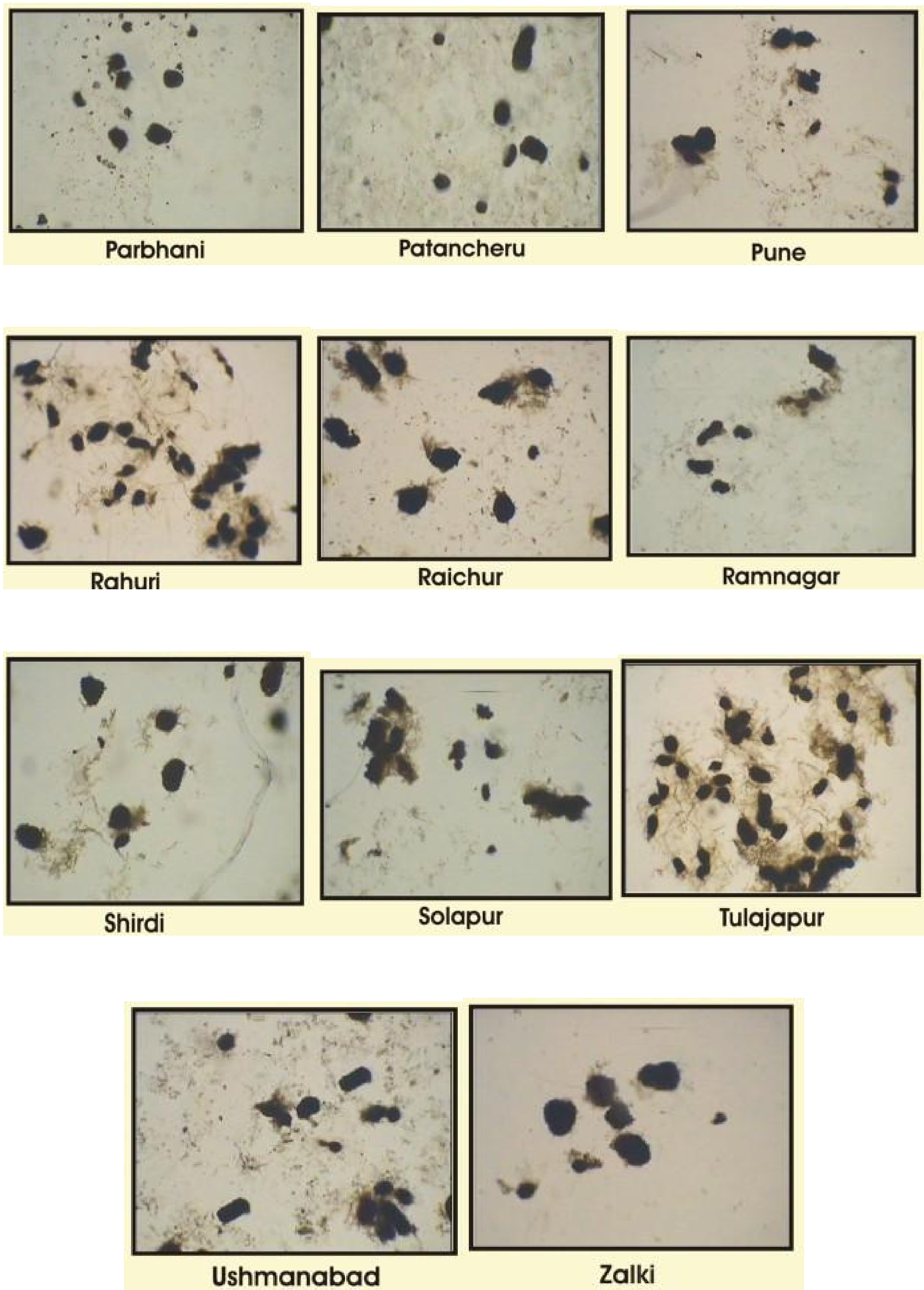


Plate2: Contd….

Table 6: Sclerotial production by the different isolates of *M. phaseolina*

Isolates	Days taken for sclerotial body formation	Number of sclerotia/microscopic field
Ri	2	42.3
Bj	4	48.6
Ga	2	45.0
Gu-1	2	50.0
Gu-2	3	49.3
Dh	2	42.3
Bo	3	47.3
At	2	44.0
Rm	3	49.6
Pa	2	48.3
Am	2	41.3
Za	2	44.6
Mu	3	42.6
So	2	39.6
Tu	3	45.0
Us	3	40.0
Pu	2	41.6
Ah	3	41.0
Ko	3	55.6
Ra	2	60.3
Sh	2	52.3
Pr	3	52.6
Na	2	51.6
Ja	3	51.0
Nz	2	46.6
Ak	3	54.3
SE m±		0.510
CD at 1%		1.92

Table 7: Number of sclerotia per 9 mm disc produced by isolates of *M. phaseolina* at different intervals

Isolates	Number of sclerotia per 9 mm disc		
	2 DAI	**4 DAI**	**6 DAI**
Ri	60	79	105
Bj	74	93	128
Ga	71	89	118
Gu-1	78	110	140
Gu-2	70	95	130
Dh	61	76	103
Bo	70	92	123
At	63	82	113
Rm	75	99	135
Pa	72	94	125
Am	59	74	101
Za	65	80	110
Mu	61	80	110
So	50	79	100
Tu	69	85	115
Us	57	72	100
Pu	60	75	102
Ah	57	73	100
Ko	90	120	155
Ra	110	135	180
Sh	85	115	149
Pr	86	118	150
Na	83	115	145
Ja	80	110	143
Nz	70	90	120
Ak	91	125	153

DAI – Days After Inoculation

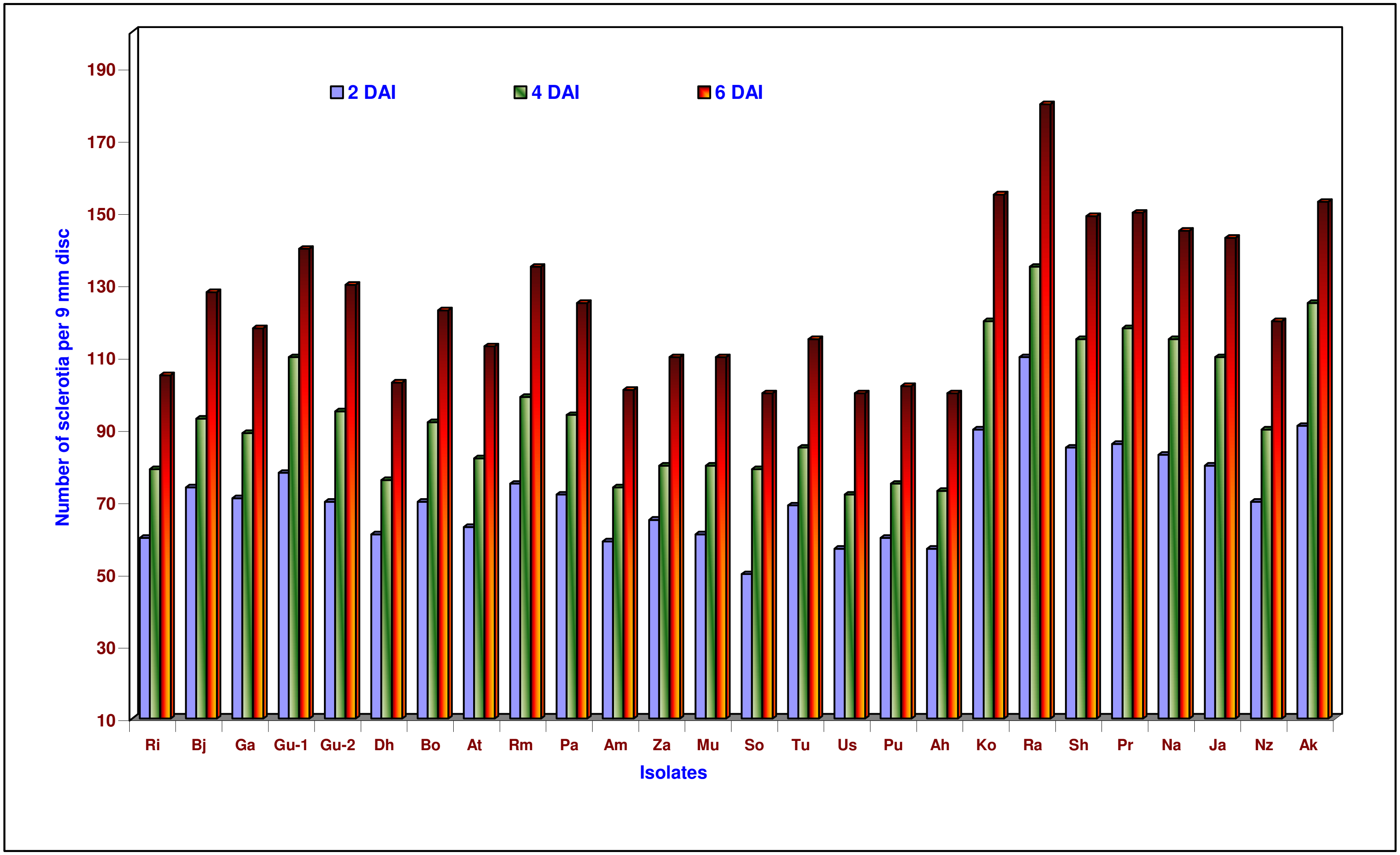


Fig. 2: Number of sclerotia per 9 mm disc produced by isolates of M. phaseolina at different intervals

Table 9: Scorable DNA bands generated with 20 RAPD primers in 26 isolates of *M. phaseolina*

Primer	Total number of bands amplified	Number of polymorphic bands produced	% polymorphism
OPA01	7	7	100.00
OPA02	8	8	100.00
OPA03	6	6	100.00
OPA04	8	8	100.00
OPA05	8	7	87.50
OPA06	15	15	100.00
OPA07	10	10	100.00
OPA08	16	15	93.15
OPA09	8	8	100.00
OPA10	14	13	92.85
OPA11	17	17	100.00
OPA12	10	10	100.00
OPA13	7	7	100.00
OPA14	8	8	100.00
OPA15	11	11	100.00
OPA16	6	5	83.33
OPA17	8	8	100.00
OPA18	10	10	100.00
OPA19	6	6	100.00
OPA20	6	6	100.00

Table 8: Length and breadth of sclerotia produced by isolates of *M. phaseolina*

Isolates	Measurement	
	Range (µm)	Average (µm)
Ri	44.3 - 93.2 x 33.8 - 58.0	64.14 x 50.00
Bj	30.0 - 59.5 x 19.2 - 41.6	45.40 x 33.58
Ga	55.4 - 88.0 x 33.0 - 48.0	69.14 x 41.30
Gu-1	47.1 - 96.8 x 32.0 - 56.6	63.98 x 46.22
Gu-2	44.0 - 77.4 x 34.5 - 56.1	53.12 x 41.72
Dh	47.4 - 58.0 x 29.7 - 42.0	53.20 x 35.40
Bo	27.0 - 45.8 x 19.2 - 27.0	36.16 x 21.96
At	40.2 - 75.0 x 33.0 - 41.2	47.80 x 35.96
Rm	38.2 - 72.1 x 25.1 - 37.6	55.98 x 30.80
Pa	40.5 - 54.1 x 34.1 - 42.0	46.56 x 37.30
Am	45.3 - 68.1 x 16.6 - 49.6	50.70 x 35.30
Za	49.1 - 86.0 x 31.3 - 62.6	69.50 x 51.24
Mu	38.6 - 68.1 x 16.6 - 45.6	51.50 x 34.50
So	60.8 - 88.0 x 32.4 - 50.0	69.92 x 39.32
Tu	35.4 - 50.2 x 27.5 - 37.0	41.86 x 31.90
Us	57.2 - 98.8 x 32.2 - 71.7	72.84 x 47.06
Pu	44.3 - 94.1 x 25.6 - 45.9	61.00 x 38.60
Ah	41.8 - 57.0 x 26.6 - 39.0	46.42 x 33.26
Ko	41.0 - 79.1 x 30.0 - 60.7	59.06 x 44.08
Ra	32.9 - 87.8 x 31.3 - 49.9	56.54 x 40.30
Sh	51.9 - 73.2 x 40.0 - 50.4	59.10 x 45.26
Pr	37.1 - 76.9 x 21.0 - 43.0	55.38 x 33.92
Na	72.0 - 103.5 x 35.0 - 60.0	83.72 x 47.44
Ja	38.2 - 65.5 x 31.6 - 47.0	50.00 x 37.96
Nz	38.9 - 65.9 x 33.2 - 38.1	48.20 x 36.42
Ak	43.8 - 111.8 x 31.1 - 84.0	85.14 x 62.72

Table 10: Similarity co-efficients based on RAPD analysis using 20 primers in 26 isolates of *M. phaseolina*

	Mu	Dh	Pu	Ja	Pa	Gu-1	Ko	Bj	So	Sh	Rm	Pr	Ri	Am	Tu	At	Ah	Ra	Ak	Ga	Nz	Us	Na	Za	Gu-2	Bo
Mu	1.00																									
Dh	0.43	1.00																								
Pu	0.45	0.38	1.00																							
Ja	0.37	0.41	0.63	1.00																						
Pa	0.24	0.37	0.39	0.61	1.00																					
Gu-1	0.32	0.34	0.34	0.47	0.56	1.00																				
Ko	0.28	0.40	0.39	0.37	0.52	0.45	1.00																			
Bj	0.26	0.36	0.29	0.26	0.28	0.35	0.20	1.00																		
So	0.23	0.34	0.34	0.43	0.41	0.36	0.32	0.42	1.00																	
Sh	0.20	0.35	0.38	0.50	0.50	0.41	0.40	0.33	0.55	1.00																
Rm	0.27	0.20	0.34	0.32	0.36	0.22	0.36	0.16	0.33	0.40	1.00															
Pr	0.20	0.27	0.28	0.25	0.30	0.27	0.23	0.37	0.38	0.31	0.36	1.00														
Ri	0.21	0.27	0.32	0.35	0.31	0.27	0.24	0.13	0.26	0.40	0.41	0.38	1.00													
Am	0.29	0.36	0.28	0.34	0.37	0.33	0.27	0.31	0.32	0.39	0.33	0.42	0.48	1.00												
Tu	0.31	0.38	0.28	0.31	0.23	0.15	0.20	0.20	0.37	0.26	0.33	0.32	0.29	0.36	1.00											
At	0.21	0.22	0.36	0.29	0.23	0.25	0.21	0.25	0.25	0.29	0.36	0.32	0.34	0.32	0.47	1.00										
Ah	0.27	0.32	0.27	0.32	0.40	0.26	0.23	0.38	0.36	0.27	0.29	0.30	0.27	0.37	0.35	0.40	1.00									
Ra	0.17	0.26	0.16	0.19	0.21	0.31	0.19	0.32	0.40	0.21	0.16	0.25	0.20	0.31	0.28	0.21	0.54	1.00								
Ak	0.22	0.32	0.27	0.32	0.45	0.34	0.28	0.23	0.33	0.32	0.29	0.35	0.32	0.35	0.40	0.28	0.50	0.44	1.00							
Ga	0.28	0.21	0.25	0.31	0.41	0.36	0.26	0.21	0.21	0.23	0.25	0.29	0.29	0.32	0.30	0.16	0.31	0.28	0.51	1.00						
Nz	0.23	0.26	0.27	0.22	0.33	0.36	0.35	0.24	0.28	0.25	0.33	0.21	0.24	0.28	0.32	0.46	0.35	0.34	0.31	0.33	1.00					
Us	0.12	0.20	0.21	0.24	0.31	0.34	0.22	0.19	0.30	0.29	0.26	0.22	0.22	0.28	0.30	0.28	0.21	0.29	0.29	0.22	0.47	1.00				
Na	0.22	0.32	0.35	0.38	0.37	0.35	0.25	0.20	0.31	0.38	0.27	0.14	0.28	0.29	0.21	0.18	0.30	0.24	0.40	0.23	0.39	0.51	1.00			
Za	0.21	0.22	0.23	0.27	0.28	0.22	0.25	0.22	0.25	0.27	0.26	0.18	0.20	0.15	0.23	0.17	0.21	0.11	0.29	0.28	0.19	0.29	0.39	1.00		
Gu-2	0.24	0.43	0.30	0.38	0.36	0.35	0.21	0.27	0.37	0.33	0.32	0.29	0.28	0.31	0.42	0.39	0.43	0.35	0.43	0.32	0.42	0.41	0.40	0.35	1.00	
Bo	0.27	0.34	0.24	0.27	0.38	0.28	0.27	0.31	0.25	0.32	0.45	0.35	0.36	0.32	0.35	0.30	0.35	0.21	0.42	0.35	0.29	0.36	0.44	0.42	0.53	1.00

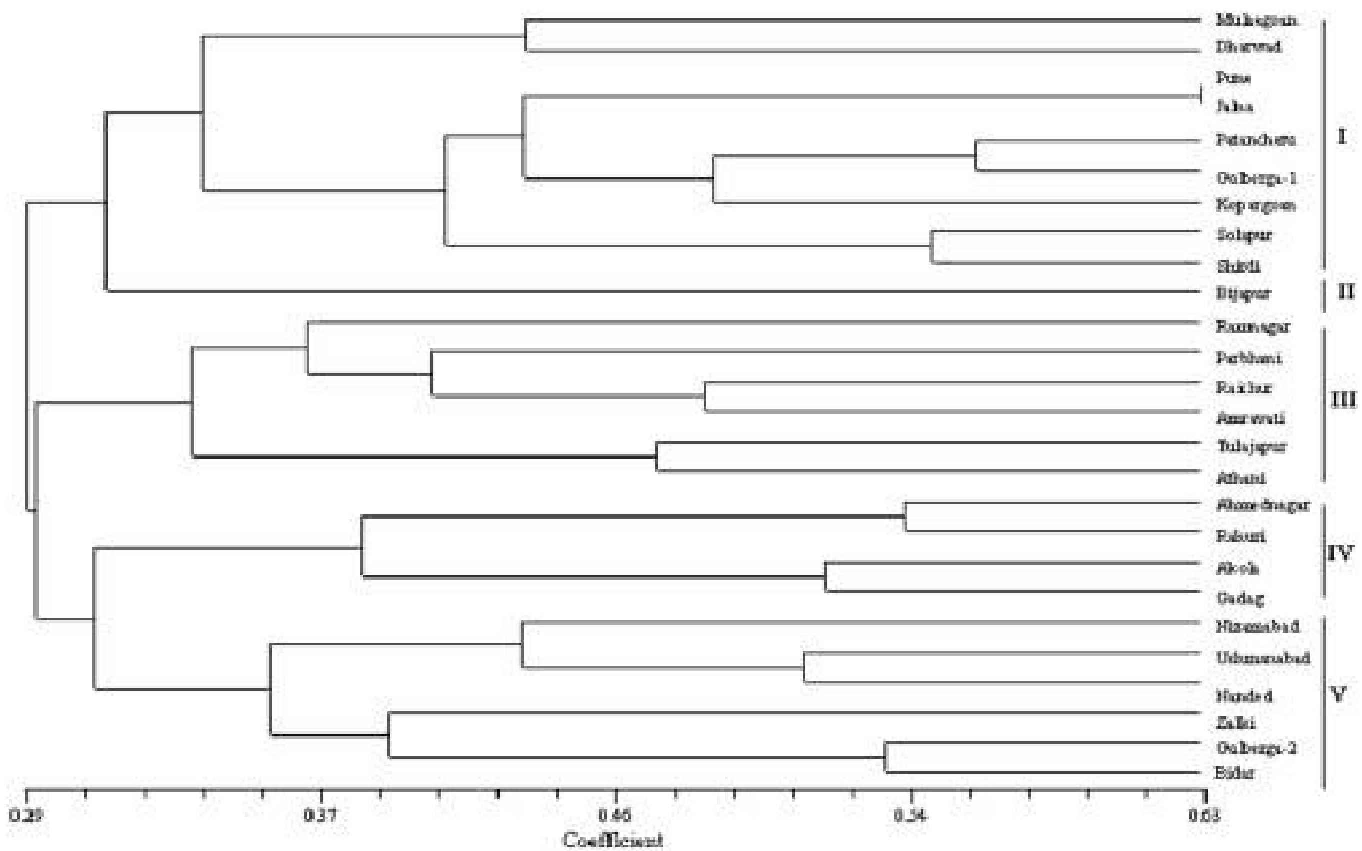


Fig. 3 : Dendrogram based on RAPD analysis of 26 isolates of *M. phaseolina*

Fig 3: Dendogram based on RAPD analysis of 26 isolates M. phaseolina

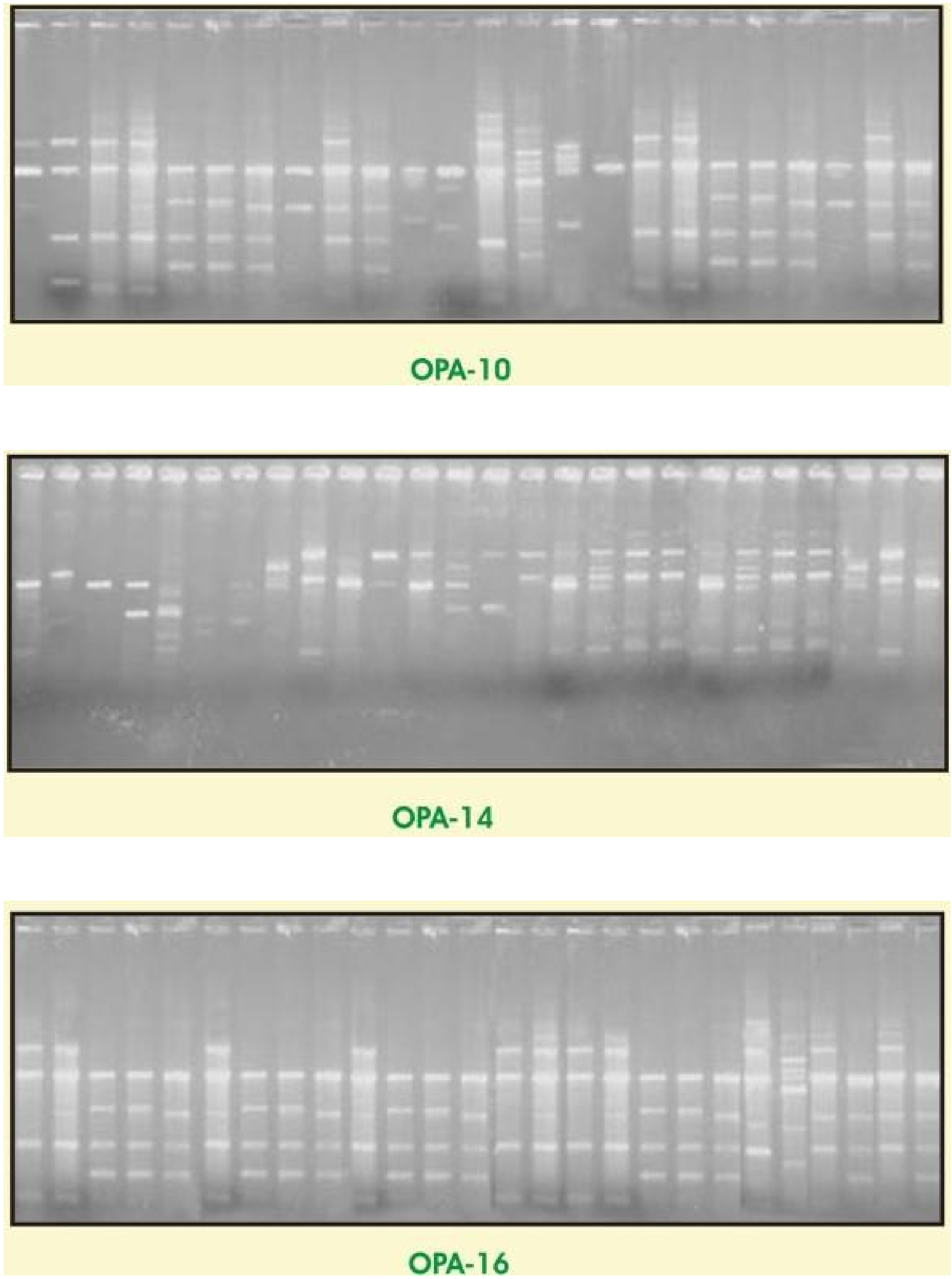


Plate 3: Randomly amilified polymorphic DNA of M. phseolina isolates

4.3 Analysis of variance for 3 traits in RIL population derived from IS22380 × E36-1

Analysis of variance revealed highly significant differences among RILs for two characters such as per cent lodging and length of spread except third character studied such as number of internode crossed (Table 11). Variability and other genetic parameters were estimated. Range, mean phenotypic and genotypic coefficient of variation (PCV and GCV), heritability estimates and predicted genetic advance as per cent of mean of characters studied are presented in Table 12. Phenotypic correlation co-efficient (PCC) and genotypic correlation co-efficient (GCC) are presented in Table 13 and 14, respectively.

The graphical presentation of PCV with GCV and heritability with genetic advance as per cent of mean for all the 3 traits are given in Fig. 4 and 5, respectively.

4.3.1 Charcoal rot disease scoring parameters

4.3.1.1 Lodging per cent

There existed a wide range of variability for this trait from 0 to 100 per cent with a grand mean of 36.49 per cent lodged plants. The high estimates of PCV (108.97%) and GCV (104.29%) were recorded. High heritability (91.6) per cent with genetic advance of 205.6 over per cent of mean were recorded.

4.3.1.2 Length of spread

A wide range of mean values between 1 cm and 23.67 cm, length of spread with a grand mean of 13.51 cm was recorded for this trait. The PCV of 33.19 per cent and GCV of 28.41 per cent were recorded. There existed a high heritability of 73.30 per cent with genetic advance of 50.11 over per cent of mean.

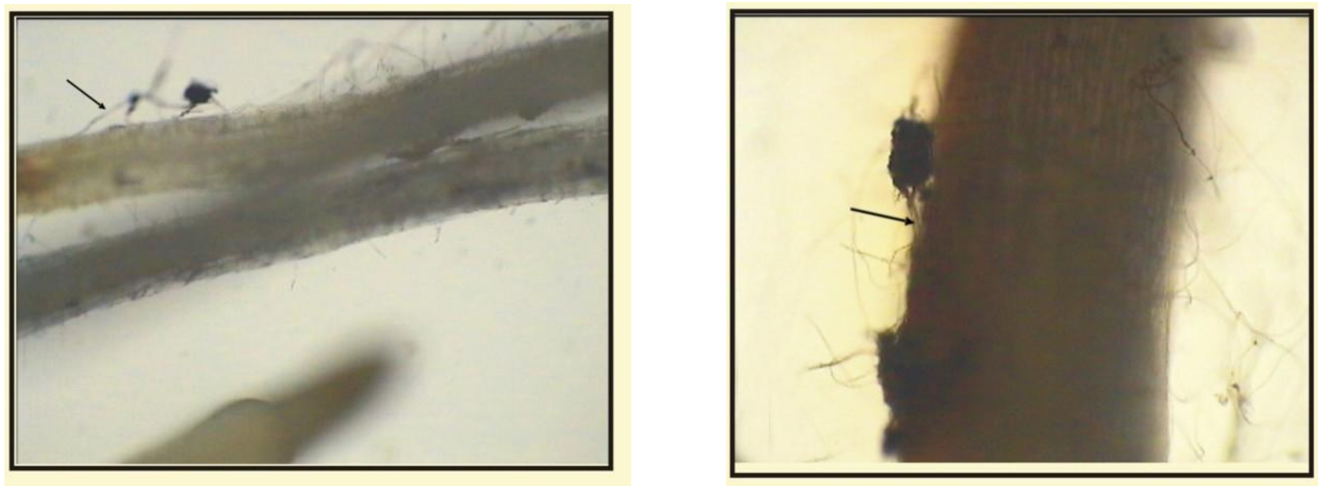
4.3.1.3 Number of internodes crossed by pathogen

The range of variation recorded was between 0.33 and 4.33 with a mean value of 2.42 internodes crossed. The phenotypic and genotypic co-efficients of variability for this trait were 37.65 and 4.24, respectively. Low heritability of 1.3 per cent with genetic advance of 0.82 per cent was exhibited by this character.

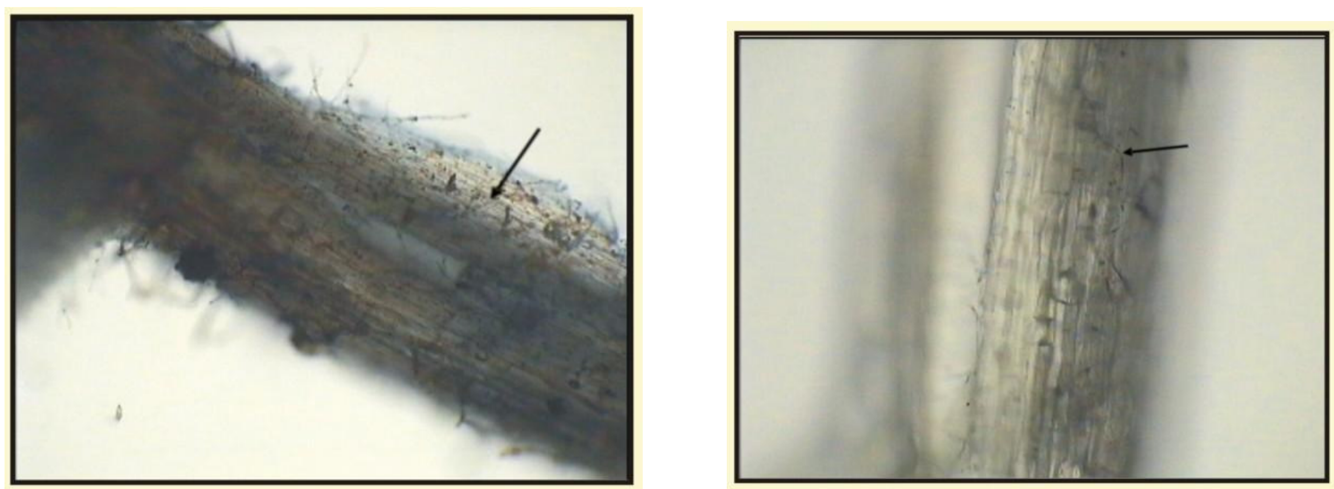
The phenotypic correlation co-efficient are presented in Table 13. The length of spread showed significant positive association with number of internodes crossed (0.700).

The genotypic correlation co-efficient are presented in Table 14. The per cent lodging exhibited significant positive association with number of internode crossed (0.940). Length of spread exhibited significant positive association with number of internode crossed.

Correlation co-efficient between charcoal rot parameters are presented in Table 15. The length of spread showed significant positive correlation with number of internode crossed (0.812).



A. Sclerotia on the surface of the root



B. Movement of hyphae inside the root

Plate 4: Roots of sorghum seedlings infected by M.phaseolina in both seceptible (SPV-86) and resistant (E36-1) varities

Plate 5: RIL population (IS22380 x E 36-1) with parents before inoculation

A. M. phaseolina cultured on tooth picks using honey peptone medium

B. Tooth picks introduced obliquely at second internode

Plate 6: Tooth pick method of inoculation

Characteristic lodging at the second Internodal region

Reaction of RIL population (Is22380 x E 36-1) for charcoal rot incidence

Pith discolouration

Plate 7: Characteristic symptoms of charcoal rot

Table 11: Analysis of variance for 3 traits in RIL population derived from IS22380 × E36-1

Source of variation	df	Mean sum of square		
		Per cent lodging	Length of spread	Number of internodes crossed
Replication	1	842.10	161.17	20.23
Genotypes	94	3029.48**	34.88**	0.843
Error	94	132.88	5.38	0.822
SE m±		11.52	2.32	0.90
CD at 5%		32.25	6.49	2.52
CD at 1%		42.68	8.59	3.33

Table 12: Variability parameters studied in 3 traits of 93 RILs derived from IS22380 × E36-1

Trait	Range		Grand mean	PCV (%)	GCV (%)	Heritability (%)	GA as (%) of mean
	Min.	Max.					
Per cent lodging	0	100	36.49	108.97	104.29	91.6	205.6
Length of spread	1	23.67	13.51	33.19	28.41	73.3	50.11
Number of internodes crossed	0.33	4.33	2.42	37.65	4.24	1.3	0.82

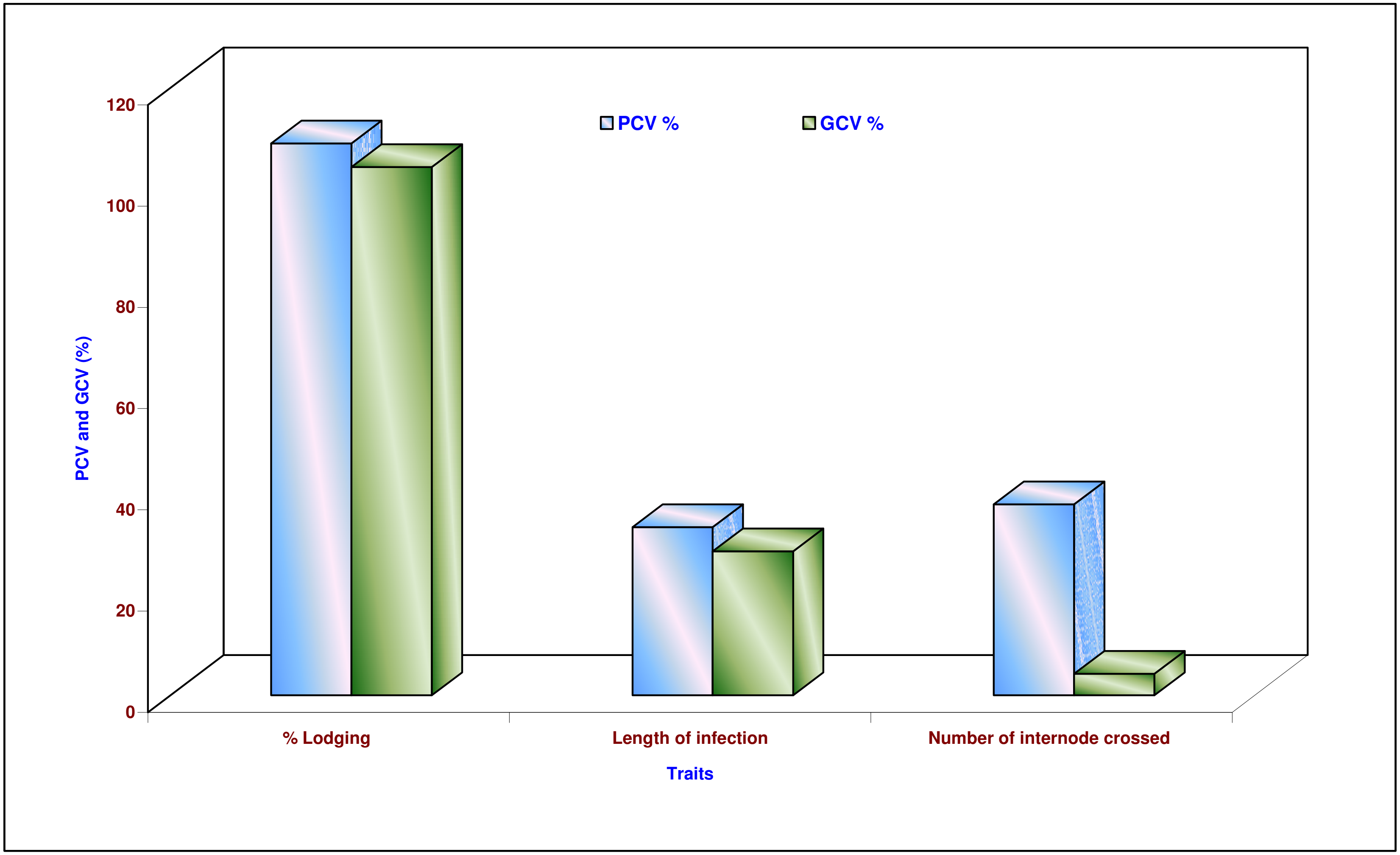


Fig. 4: PCV and GCV of different traits in RILs of IS22380 x E36-1

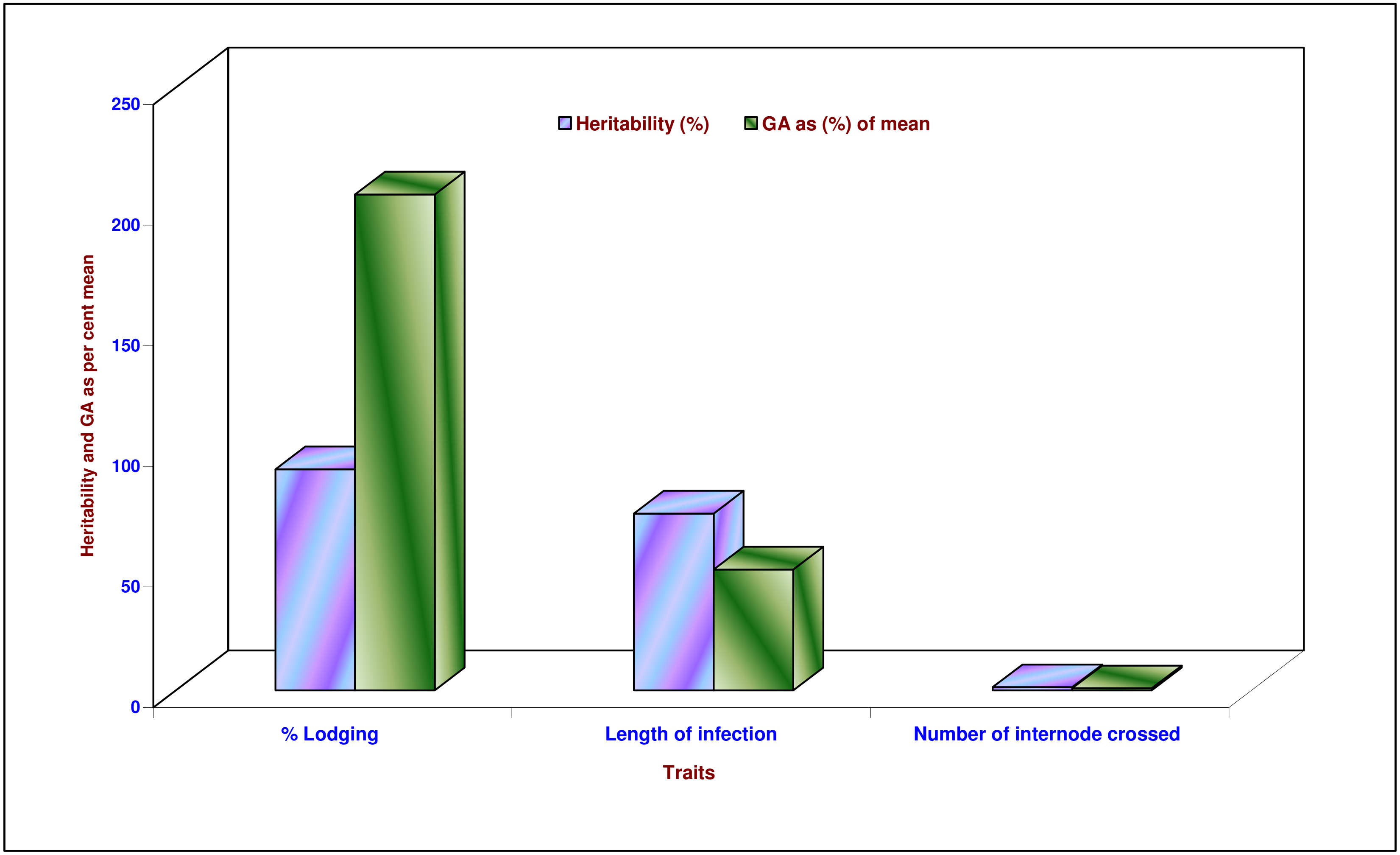


Fig. 5 : Heritability and GA as per cent mean of different traits in RILs of IS22380 x E36-1

Table 13: Phenotypic correlation coefficient

Traits	Per cent lodging	Length of spread	Number of internodes crossed
Per cent lodging	1.000	0.082	0.124
Length of spread		1.000	0.700**
Number of internodes crossed			1.000

** Significant at the 0.01 level

Table 14: Genotypic correlation coefficient

Traits	Per cent lodging	Length of spread	Number of internodes crossed
Per cent lodging	1.000	0.120	0.940**
Length of spread		1.000	0.898 **
Number of internodes crossed			1.000

** Significant at the 0.01 level

Table 15: Correlation co-efficient between charcoal rot parameters

Traits	Per cent lodging	Length of spread	Number of internodes crossed
Per cent lodging	1		
Length of spread	0.99	1	
Number of internodes crossed	0.169	0.812**	1

**Correlation is significant at the 0.01 level.

5. DISCUSSION

Sorghum is the fourth most important cereal after wheat, rice and maize and is a staple food for more than three hundred million people living in Asia and Africa besides, being fodder for animals.

Sorghum is susceptible to several diseases, among them, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid., has attained a serious status in India, in general and Karnataka and Maharashtra in particular. Currently both cultivated varieties and hybrids are susceptible when moisture stress coupled with high soil temperature prevails during grain filling period. Continuous epiphytotics were observed during 1977-80 in both *kharif* and *rabi* seasons and it was more in *rabi* season. During 1977, epiphytotics of this disease, resulted in 48 per cent loss in grain weight (Anahosur and Rao, 1977) and during 1990-91, it appeared in very severe form at Dharwad (Karnataka) resulting in 100 per cent lodging in several genotypes in sick soil (Hiremath and Palakshappa, 1994).

Charcoal rot resistance is a quantitative trait, involving number of components, which are influenced by environment. Phenotype based selection for this trait is difficult.

Hence, present investigation was undertaken to study the morphological variation and molecular characterization of different isolates, investigate the path of movement and to map the variability of charcoal rot resistance to D5 isolate among 93 RILs developed by crossing IS22380 × E36-1.

5.1 Collection of samples and isolation of *M. phaseolina*

Twenty six charcoal rot infected sorghum plant samples were collected from selected agricultural research stations in Karnataka (Dharwad, Gadag, Athani, Raichur, Gulberga, Bidar and Bijapur); Maharashtra (Zalki, Kopargoan, Pune, Shirdi, Rahuri, Ahmednagar, Parbhani, Mulegoan, Solapur, Tulajapur, Ushmanabad, Amaravati, Jalna, Nanded and Akola) and Andhra Pradesh (Ramnagar, Patancheru and Nizamabad).

Isolation of the fungus was made by following standard isolation procedure and the fungus was confirmed with respect to the morphological characters described by Ashby (1927) and Goidanich (1947) and identified as *M. phaseolina*.

The charcoal rot pathogen isolated from the infected samples of each location was considered as an isolate of respective location. The names of isolates were designated by considering the location from which the infected sorghum plant samples were obtained.

5.1.1 Morphological variability

Inherent ability of different isolates to grow on potato dextrose agar medium could vary and association of such a trait to any of pathological properties of pathogen either in *M. phaseolina* or other fungi is seldom exists in literature. However, isolates with ability to grow faster will have advantage in terms of population built up for causing disease.

Morphological characters on potato dextrose agar media showed variation among 26 isolates of *M. phaseolina*. The cultures could be assigned to four groups *viz.,* Dh, Us and Pr isolates produced grayish white colour; Ri, Ga, Gu-1, Gu-2, Bo, Rm, Za, So, Pu, Ah, Ko, Na, Ja, Nz and Ak isolates produced blackish gray colour; Bj, At, Am, Mu, Tu and Ra isolates produced deep black colour and Pa and Sh isolates produced cottony white colour. The isolates also could be assigned to two groups on the basis of texture ; fluffy colony produced by Dh, Am, Sh and Pr isolates and flat colony produced by Ri, Bj, Ga, Gu-1, Gu-2, Bo, At, Rm, Za, Mu, So, Tu, Us, Pu, Ah, Ko, Ra, Na, Ja, Nz and Ak isolates. Shekhar *et al.* (2006a) made similar observations in case of Bangalore and Arabhavi isolates of *M. phaseolina,* where in they produced grayish white colour; Udaipur and Hyderabad isolates produced blackish gray, Ludhiana and Delhi isolates produced deep black center with creamish periphery and Coimbatore isolate produced cottony white colour. Similarly Coimbatore isolate produced thick fluffy growth and Delhi and Ludhiana isolates produced less fluffy growth.

Mean colony diameter among 26 isolates varied considerably. A higher mean colony diameter was observed in Ri, Ga, Gu-2, Am, Mu, So, Ah, Ko, Sh, Na and Nz isolates and a significantly lower mean colony diameter was observed in Bj and Tu isolates. This property of an isolate indirectly reflects the intrinsic ability to grow faster. However, observed property on PDA really reflects the natural variation remains to be elucidated in *M. phaseolina.*

Grouping of isolates based on mean colony diameter four days after inoculation on PDA revealed four groups. The first group included 18 isolates with a spread of 80.37 to 90.00 mm, the last group included only three isolates Bj, Za and Tu with a spread in the range of 51.45 to 61.08 mm. Similar observations among the isolates was made by Subramaniam (1994), where in first group included 28 isolates with a spread of 71.26 to 90.00 mm, while the second group (71.25-53.52 mm) included only two isolates Bj3 and Bj4 with a mean growth of 67.66 mm and 60.00 mm, respectively, indicating their slow nature of growth.

Regarding size of sclerotia, it was observed that the Rm isolate produced largest sclerotia (94.0 µm), while the smallest sized sclerotia (30.0 µm) was recorded in Bj isolate. The round shaped sclerotia were observed in majority of isolates *viz.,* Ri, Bj, Ga, Gu-1, Gu-2, At, Pa, Za, Mu, Tu, Pu, Ah, Ko, Sh, Na, Ja, Nz and Ak isolates. Oblong shape of sclerotia were observed in Dh, Bo, Rm, Am, So, Us, Ra and Pr isolates. Similar observations were reported by Shekhar *et al.* (2006a), that the size of sclerotia, varied from 95.7 µm to 66.9 µm throughout the study. It was observed that the Hyderabad isolate produced largest sclerotia of 95.7 µm followed by Bangalore, Udaipur, Ludhiana, Delhi, Arabhavi, while the smallest sized sclerotia of 66.9 µm was noticed in Coimbatore isolate. Similarly Udaipur, Bangalore, Coimbatore isolates had oblong shape whereas the other one had round shape sclerotia.

Average sclerotial size revealed 8 groups, with Rm isolate recording maximum size (94.0 µm) and Bj isolate recording the lowest (30.0 µm). Several workers (Goidanich and Camici, 1947; Dhingra and Sinclair, 1973b; Ghosh and Sen, 1973; Raut and Ingle, 1989; Waseer *et al.,* 1990; Subramaniam, 1994 and Shekhar *et al.,* 2006a) have reported variation in size and shape of sclerotial bodies of different isolates of *M. phaseolina*.

Haigh (1928), grouped isolates based on average sclerotial size, while Arca and Yildizn (1989) grouped isolates based on number of sclerotial bodies. Anilkumar and Sastry (1980), reported that an ability of the isolate to produce sclerotia changed with the growth media. Waseer *et al.* (1990) reported that the sclerotial size of soybean isolate on PDA was different from that of other media, though, was found to be within the range described for *M. phaseolina*. Subramaniam (1994) reported that the average sclerotial size revealed 18 groups.

The number of sclerotia/microscopic field 10 X was maximum of 60.3 per microscopic field in Ra isolate, while minimum number was observed in So (39.6/microscopic field).

Similar observations were made by Shekhar *et al.* (2006a) whereon maximum numbers of sclerotia/microscopic field (52.0) in Hyderabad isolate while minimum number was observed in Coimbatore isolate (44/microscopic field).

Majority of the isolates took 2-3 days for sclerotial formation, except Bj isolate, which took four days for sclerotial formation. Such variations, in days required for sclerotial formation has been reported by Anilkumar and Sastry (1980), who observed that, the sunflower isolates took least time (2 to 3 days for sclerotial formation), while the brinjal and cowpea isolates took 4 to 10 days depending on the media used. Subramaniam (1994) also noticed that most of the isolates took 3-4 days for sclerotial formation, except oil palm (OP1, OP2 and OP4), groundnut (Gn), soybean (So) and maize (Ma) isolates, which took 5-6 days for sclerotial formation.

Numbers of sclerotia/9 mm disc at different intervals were recorded. The maximum number of sclerotia/9 mm disc was observed in Ra isolate (110, 135 and 180) during 2^{nd}, 4^{th} and 6^{th} DAI, while the minimum number was observed in So (50) during 2^{nd} DAI, Us (72) during 4^{th} DAI and So, Us and Ah(100) during 6^{th} DAI. Similar observations were made by Shekhar *et al.* (2006a). They observed that Hyderabad isolate had maximum number of sclerotia/9 mm disc (180), while the minimum number was observed in Coimbatore isolate (169/9 mm disc).

Length and breadth of sclerotia produced by 26 isolates of *M. phaseolina* were analyzed among 26 isolates. Higher length and breadth was observed in Ak isolate (85.14 × 62.72 μm), while the lower length and breadth was observed in Bo isolate (36.16 × 21.96 μm).

5.1.2 Genetic variability

The suitability of random amplified polymorphic DNA (RAPD) was tested to detect the variations among the isolates of *M. phaseolina*. In the present investigation, OPA series primers were used to determine genetic distance among isolates. Of the 20 primers used for amplification OPA1, OPA2, OPA3, OPA4, OPA6, OPA7, OPA9, OPA11, OPA12, OPA13, OPA14, OPA15, OPA17, OPA18, OPA19 and OPA20 showed cent per cent polymorphism among isolates.

Information on banding pattern for all the amplified primers was used to determine genetic relatedness among isolates. Genetic similarity co-efficient ranged from 0.12 to 0.63 among 26 isolates. The maximum genetic similarity of 63 was observed between Ja and Pu, whereas, least genetic similarity was observed between Us and Mu isolates.

It was evident from the dendrogram, that the set of primers chosen for the study did not reveal any grouping of the isolates into phenotypically intended categories such as highly virulent, moderately virulent and less virulent. However all the primers generated of polymorphic DNA fragments.

Similar work was carried out by Su *et al.* (2001) employing RAPD markers to establish relationship between 45 isolates from soybean, cotton, sorghum and corn. They could clearly place isolates from various sources in specific clusters. It appears that sorghum and soybean isolates are less diverse than cotton and corn isolates. Almeida *et al.* (2003) by using four RAPD loci put 55 isolates of *M. phaseolina* from soybean into three distinct clusters. Jana *et al.* (2003) demonstrated the genetic diversity among the isolates of *M. phaseolina* obtained from different host and from different geographical regions. Rajkumar (2004) observed molecular profiling using RAPD markers indicated the genetic differences among a set of ten isolates of *M. phaseolina* representing Dharwad and Bijapur regions. Jana *et al.* (2005) employed successfully universal rice primers to cluster 40 isolates of *M. phaseolina* from soybean, cotton and chickpea. Recently Shekhar *et al.* (2006b) by using 100 RAPD primers put seven isolates of *M. phaseolina* from corn into three distinct groups.

5.2 Path of infection

Abundant hyphae of *M. phaseolina* were observed on the root surface one day after inoculation. No hyphae were observed with in the root hairs. The sclerotia formed on the surface of the roots and the pathogen penetrated the roots by 24 hr. The hyphae extended inter and intracellularly into the epidermal and cortical tissues in both susceptible and resistant varieties.

The present study confirms the earlier observations made by Chan and Sakston (1973); Ammon *et al.* (1971) with *M. phaseoli* infecting sunflower and soybean, and Karunakar *et al.* (1992) in sorghum seedlings.

5.3 Genetic variability and character association for charcoal rot disease traits in RIL population

Charcoal rot resistance is a quantitative trait, involving a number of component traits, which are influenced by G × E interaction. Phenotypic selection for such a trait will be difficult.

For analysis of charcoal rot resistance, genetic components of means and variance obtained using 93 recombinant inbred lines (RILs) evaluated along with their parents revealed variation. RILs displayed considerable differences in their mean performance with respect to all the three characters studied. Genetic variability among these RILs would be obvious as this population has been developed from deliberately selected, phenotypically distinct parents for characters, including charcoal rot resistance. Wide range of variation among line indicates utility of the RILs for genetic investigation.

The phenotypic co-efficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the characters studied, suggesting that the traits studied had high environmental influence.

High values of PCV and GCV were obtained for per cent lodging and length of spread. High values of PCV was obtained for all the 3 characters *viz.,* per cent lodging, length of spread and number of internodes crossed where as low GCV was obtained for number of internodes crossed. These results are in accordance with the results obtained by Rajkumar (2004) with same set of population.

High genotypic coefficients were observed for per cent lodging and length of spread. Similar observations were also made by Rajkumar (2004) by using the same RILS. However, GCV for number of internodes crossed were lower than reported (Rajkumar, 2004). The coefficient of variation reveals only the extent of variability present for different character in the population. Heritability is a useful quantitative parameter, which considers the role of heredity and environment determining the expression of the character (Allard, 1960). In present study, heritability ranged from 1.3 for number of internodes crossed to 91.6 for per cent lodging. The highest heritability indicates the presence of higher proportion of fixable additive variation in the population.

Presence of genetic variability in any population is a prerequisite for selection to be effective, while heritability indicate how well this can be done. Expected genetic advance is a parameter that considers both and provides a realistic estimate of gain achievable following selection. However, in present study, high heritability coupled with high per cent of mean of genetic advance over mean was observed for the characters *viz.,* per cent lodging and length of spread. Selection made through these characters would be effective as they are more likely to be controlled by additive gene effects. The results are similar to that of Bhat (1975) and Reddy *et al.* (1996) in sorghum.

Length of spread had significant correlation with number of internodes crossed. Per cent lodging and length of spread recorded significant positive association with number of internodes crossed. Among the parameters of charcoal rot resistance a highly significant and positive association was found with length of spread and number of internodes crossed at both phenotypic and genotypic level, whereas, positive and significant association between per cent lodging and number of internodes crossed was observed at genotypic level. A correlation study can provide the role of component characters to the target phenotype. In the present investigation, both genotypic and phenotypic correlations were worked out for disease parameter, to assess the extent and nature of association between component traits of disease resistance.

The length of spread showed significant positive correlation with number of internode crossed.

Future line of work

- There is need to study the variability in the isolates of *M. phaseolina* with respect to serology and biochemical analysis.
- To study the variability by using other molecular markers such as ARDRA or ITS-RFLP

6. SUMMARY AND CONCLUSIONS

Charcoal rot of sorghum caused by the fungus *Macrophomina phaseolina* is a root and stalk rot disease of potentially destructive nature particularly in post rainy season grown crop.

The present investigation included collection, morphological and molecular characterization, path of infection and assessment of the variability of charcoal rot resistance to D5 isolate in 93 RILs derived from cross IS22380 × E 36-1.

Twenty six, infected sorghum samples were collected from sorghum growing regions of Karnataka, Maharashtra and Andhra Pradesh, to assess the variability in the pathogen under *in vitro* condition. From the collected samples, (Karnataka (Dh, Ga, At, Ri, Gu-1, Gu-2, Bo and Bj), Maharashtra (Za, Ko, Pu, Sh, Ra, Ah, Pr, Mu, So, Tu, Us, Am, Ja, Na and Ak) and Andhra Pradesh (Rm, Pa and Nz) *M. phaseolania* was isolated to study the molecular variability using RAPD markers and each sample was considered as an isolate. Based on colony colour, the cultures were assigned to four groups; grayish white (Dh, Us, Pr); blackish gray (Ri, Ga, Gu-1, Gu-2, Bo, Za, So, Pu, Ah, Ko, Na, Ja, Nz and Ak); dark black (Bj, At, Am, Mu, Tu and Ra) and cottony white colour (Pa and Sh)

Isolates were also assigned into two groups, on the basis of colony texture *viz.,* fluffy colony produced by Dh, Am, Sh, Pr isolates and flat colony produced by other isolates.

Grouping of isolates based on colony diameter on PDA revealed four groups. The first group included 18 isolates falling under the range of 80.37 to 90.00 mm, while the last group included 3 isolates Bj, Za and Tu with a spread of 51.45 to 61.08 mm.

Grouping of isolates based on average sclerotial size (μm) revealed 8 groups. Rm, Am, Za, So, Us, Ak isolates recorded maximum sclerotial size ranging from 87.65 μm to 96.10 μm, while Bj, Ga, Gu-2, Dh, Ra, Sh isolates recorded lowest sclerotial size ranging from 28.29 μm to 36.76 μm.

Maximum number of sclerotia per microscopic field was observed in Ra isolate, while the minimum number was observed in So isolate.

Maximum number of sclerotia/9 mm disc was produced by Ra isolate (110, 135 and 180) during 2^{nd}, 4^{th} and 6^{th} day after inoculation, while, the minimum number was observed in So (50) during 2^{nd} DAI, Us (72) during 4^{th} DAI and So, Us and Ah (100) during 6^{th} DAI. Based on length and breadth Ak isolate produced higher length and breadth of 85.14 × 62.72 μm, while the lower length and breadth of 36.16 × 21.96 μm was noticed in Bo isolate. RAPD analysis distinguished 26 isolates into five major clusters.

Abundant hyphae of *M. phaseolina* were observed on the root surface after one day of inoculation. The sclerotia found on the surface of the roots and the pathogen penetrated the roots by 24 hr in both susceptible (SPV-86) and resistant (E 36-1) varieties.

A set of 93 RILs derived from the cross IS22380 × E 36-1 were used for phenotypic evaluation. Analysis of variance revealed highly significant difference among genotypes and parents for all the traits.

The characters like length of infection, number of internodes crossed and lodging per cent exhibited high values of GCV (genetic coefficient of variation) and PCV (phenotypic coefficient of variation). High per cent heritability coupled with high genetic advances observed for characters *viz.,* length of spread, number of internode crossed and per cent lodging. Correlation studies revealed highly significant and positive association between length of spread and number of internodes crossed at both phenotypic and genotypic level.

- On the basis of morphological variability all the 26 isolates showed variation with respect to colony colour, texture, colony diameter, sclerotial size, number of sclerotia per microscopic field, number of sclerotia per 9 mm disc, length and breadth of sclerotia.
- It is evident from dendrogram that the primers as such could not group individual isolates into phenotypically intended categories *viz.,* highly virulent, moderate virulent and low virulent.
- Analysis of variance revealed highly significant differences among genotypes and parents for all the 3 traits.

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** Originals are not seen*

MORPHOLOGICAL AND GENETIC VARIABILITY AND HOST RESISTANCE RESPONSE OF SORGHUM RECOMBINANT INBRED LINES (RILs) TO A VIRULANT ISOLATE OF *Macrophomina phaseolina* (Tassi) Goid.

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ABSTRACT

Twenty-six isolates of *Macrophomina phaseolina* causing charcoal rot of sorghum, collected from different parts of Karnataka, Maharashtra and Andhra Pradesh varied both morphologically and genetically, to understand the infection and movement, the selected virulent isolates of the pathogen was used and to map the variability of charcoal rot component traits to D_5 isolate among RILs of IS 22380 X E36-1cross was used. Colony diameter varied from 60.0 mm to 90.0 mm among isolates. On the basis of colony colour, the isolates could be divided into 4 groups such as grayish white, blackish gray, deep black and cottony white. Majority of the isolates took 2-3 days for sclerotial body formation. Ramnagar isolate produced largest size of sclerotia (94.0 µm) followed by others. On the basis of shape of sclerotia, isolates were divided in to 2 groups *viz*., round and oblong shape. Rahuri isolate produced highest number of sclerotia (60.3 sclerotia/microscopic field 10X) and 110, 135 and 180 sclerotia/9 mm disc during 2^{nd}, 4^{th} and 6^{th} day after inoculation. Molecular profiling using randomly amplified polymorphic DNA (RAPD) markers revealed high level of the genetic diversity among the isolates of *M. phaseolina.* Similarity coefficient and the resulting phenotypic tree was analyzed vis-à-vis morphometry and geographical origin of isolates maximum genetic similarity of 63 was observed between Jalna and Pune isolates.

Root samples of sorghum seedlings inoculated with *M. phaseolina* were collected at intervals upto 10 days. Necrotic lesions were developed on roots 48 h after inoculation. Abundant hyphae were observed on the surface of root after 24 h of inoculation. To evaluate the charcoal rot incidence, 93 Recombinant Inbred Lines (RILs) derived from cross between IS22380 and E36-1 were evaluated. The contrasting behaviour of parents for charcoal rot resistance showed wide range of variability.