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Review Article

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Biology, Epidemiology and Management of the Pathogenic Fungus *Macrophomina phaseolina* (Tassi) Goid with Special Reference to Charcoal Rot of Soybean (*Glycine max* (L.) Merrill)

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Abstract

The fungus *Macrophomina phaseolina* is a causative agent of diseases in more than 500 plant species. The fungus is primarily soil-inhabiting but is also seed-borne in many crops including soybean. It survives in the soil mainly as microsclerotia that germinate repeatedly during the crop-growing season. Low C : N ratio in the soil and high bulk density as well as high soil moisture content adversely affect the survival of sclerotia. The disease can be managed to some extent by cultural practices, organic amendments, seed treatment and genetic host resistance. The scattered literature on these aspects is reviewed in this paper.

Introduction

Soybean (*Glycine max* (L.) Merrill) is an important oilseed crop. The total soybean production in the world was projected to be 250.39 million metric tonnes in 2009–10 (USDA 2009). The USA, Brazil, Argentina, China and India, in order of their share in total production, are the major soybean-producing countries. In India, soybean is the largest oilseed crop, which is estimated to presently cover 9.21 m ha producing 9.81 m t of soybean (DAC, GOI 2010). Initially, the crop was relatively free of pests but its rapid expansion and continuous cultivation coupled with altered climatic conditions led to the appearance of number of soybean diseases in different regions of the world.

Soybean is an important host of the fungus *Macrophomina phaseolina* (Tassi) Goid that causes charcoal rot, dry root rot, dry weather wilt, ashy stem blight and seedling blight disease in over 500 plant species (Su et al. 2001). *Macrophomina phaseolina* is a soil-inhabiting organism capable of infecting soybean at any crop growth stage, but usually, it infects at post-flowering stage. The fungus is also seed-borne in many crops including soybean. It produces microsclerotia in root and stem tissues of host plants, which enable it to survive in soil for 2–15 years and act as primary source of inoculum (Meyer et al. 1974). There is equivocal evidence for host plant specificity in *M. phaseolina* (Zazzerni and Tosi 1989).

In India, the charcoal rot, which is used to be a minor disease of soybean until 2004, became a serious pest due to altered weather conditions particularly on the account of longer drought spells during crop growth period. Subsequently, the disease appeared intermittently at high levels in some areas, causing substantial yield losses. The disease can be managed to some extent by cultural, chemical and biological methods (Bristow and Wyllie 1975; Gupta 2004). To date, no charcoal rot-resistant variety of soybeans is available. However, variation in root tissue colonization by *M. phaseolina* among soybean genotypes was reported by many researchers (Bristow and Wyllie 1984; Pearson et al. 1984; Smith and Carvil 1997; Kendig et al. 2000; Paris et al. 2006; Mengistu et al. 2007; Wrather et al. 2008; Talukdar et al. 2009).

There has been considerable research into this pathogen; yet, few efforts have been made to consolidate the scattered literature. Here, we critically review the available literature on *M. phaseolina* with special reference to charcoal rot of soybean.

Taxonomy and Nomenclature

Macrophomina phaseolina (Tassi) Goid. (= *Tiarosporella phaseolina* (Tassi) Van der Aa) belongs to the anamorphic Ascomycetes (Ndiaye 2007). The pycnidial state of the fungus was originally named *Macrophoma phaseolina (*Tassi 1901) subsquently named *Macrophoma phaseoli* (Maublanc 1905) and then *Macrophomina phaseoli* (Ashby 1927). Finally, the name *Macrophomina phaseolina* was settled upon (Goidanich 1947).

An unconfirmed report (Mihail 1992) of a teleomorph of *M. phaseolina* naming it as *Orbilia obscura* (Ghosh et al. 1964) is also available. The microsclerotial state of the fungus was described as *Rhizoctonia bataticola* (Taub.) Butler on *Ipomoea batatas* (Halsted 1890). The same fungus was isolated from cowpea in India in 1912 by Shaw (Dhingra and Sinclair 1978) and was then named *Sclerotium bataticola*. Despite the teleomorph being unknown in this pathogen, *M. phaseolina* is a member of the family Botryosphaeriaceae (Crous et al. 2006).

Host Range and Distribution

Macrophomina phaseolina is a pathogen of crops like soybean, common bean, mungbean, sorghum, maize, cotton, peanut, sesame, cowpea, chickpea and clusterbean (Dhingra and Sinclair 1977; Lodha et al. 1986; Diourte et al. 1995). Softwood and other forest trees such as *Abies*, *Pinus*, *Pseudotsuga, Cassia* (Lodha et al. 1986; McCain and Scharpf 1989), fruit trees (*Citrus* spp., *Cocos nucifera*, *Coffea* spp., *Ziziphus mauritiana, Leucaena* spp.), medicinal plants and weed species (Lodha et al. 1986; Songa and Hillocks 1996) are also hosts. It is known to occur on soybean in North and South America, Australia, Asia, Europe and African continents (McGee 1991). In the USA, it usually occurs in Missouri, Mississippi, Alabama, Illinois and Indiana (Wyllie 1988). In India, it commonly occurs in the states of Madhya Pradesh, Maharashtra, Rajasthan and Delhi (Gupta and Chauhan 2005).

Symptoms

Symptoms on seedlings

Seedlings can be infected in years when soils are exceptionally dry and soil temperatures are continuously above 35°C for 2–3 weeks. After emergence, symptoms can be visible on cotyledons as brown to dark spots. Sometimes, the margins of the cotyledons become brown to black and shed at an early stage. From the unifoliate leaf stage onwards, the symptoms appear on emerging hypocotyls of infected seedlings as circular to oblong, reddish-brown, lesions that may turn dark brown to black after several days. These lesions may extend up the stem. Infected seedlings may die if hot and dry conditions persist.

Symptoms on adult plants

The first aboveground symptoms appear between 1 and 4 weeks before normal maturity. The pathogen causes lesions on the roots, stems, pods and seeds. From ground level upwards, superficial lesions, light brown to grey in colour, infrequently appear on the stem. Microsclerotia are formed in the vascular tissues and in the pith, giving a greyish-black appearance to the subepidermal tissues of the stem. Such discolouration is first visible at nodes as profuse small, black, randomly distributed specks. A twin stem abnormality is usually observed in greenhouse infections (Bristow and Wyllie 1986). Foliar symptoms progress from top of the plant downwards. Leaves of infected plants remain smaller than normal and subsequently turn yellow prior to wilting (Gupta and Chauhan 2005). A reddish-brown discolouration of the vascular elements of roots and lower stem precedes the premature yellowing as the fungus spreads up the stem during the season. The infected mature and dry pods are covered with locally or widely distributed black bodies (microsclerotia). The fungus penetrates the pods and grains, inducing diverse symptoms. Diffuse black spots or blemishes appear on the seeds. Microsclerotia sometimes are produced in fissures and cracks in the seed coat. After the death of the plant, numerous, minute, pinhead-sized microsclerotia appear, which can be seen readily when the epidermal tissue of the lower stems and roots is peeled from the affected parts. The infected crop in the field exhibits premature yellowing in scattered patches. Under severe disease conditions, the crop over a large area in the field may be affected. Normally, in the infected crop, the dead leaves remain attached to the petiole for several days after death.

Yield Loss

The estimated yield loss due to charcoal rot in soybean in the top 10 soybean-producing countries during 1994 was 1.234 million metric tonnes (Wrather et al. 1997). Average annual losses were estimated to be 5% in Missouri (USA) with some growers experiencing 30–50% loss (Wyllie 1988). Charcoal rot was responsible for greater losses in soybean in comparison with other diseases from Central Mississippi and Alabama to Central Illinois and Indiana (Moore 1984). A severe epidemic of charcoal rot of soybean was reported in Iowa during the 2003 growing season (Yang and Navi 2005) and it was ranked second on the list of diseases that suppressed soybean yield during 2003 in USA. (Wrather and Koenning 2006). Estimates of soybean yield suppression due to charcoal rot in the United States were 1.98 million metric tons in 2003, 0.28 lakh ton in 2004 and 0.49 lakh ton in 2005 (Wrather and Koenning 2006).

In India, epiphytotics occur in areas where temperature ranges from 35–40°C during the crop season and the disease can cause up to 80% yield losses. During the 1997 season, charcoal rot caused substantial loss to plant stand and yield in soybean in Guna District of Madhya Pradesh State (Gupta and Chauhan 2005).

Isolate Variability and Pathogen Population

While *M. phaseolina* is a polyphagous pathogen, there is no evidence of host specificity (Maia et al. 2004). Hildebrand et al. (1945) indicated that two strains (Ontario and Texas) of *Macrophomina phaseoli* (= *M. phaseolina*) could be distinguished on the basis of differences in the size and number of microsclerotia. Dhingra and Sinclair (1973) observed that the isolates varied from virulent to moderately virulent to weakly virulent. They also observed a general correlation between *in vitro* growth on potato dextrose agar (PDA) and virulence of the isolates but no variation in virulence within any single isolate. Ahmed and Ahmed

(1969) observed that cultural characteristics and growth rates of eight different jute isolates of *M. phaseolina* appeared to be related to their pathogenicity. The colour of cultures on PDA, the ability to sporulate in infected host plants and pycnidial size have also been reported to vary greatly (Dhingra and Sinclair 1978). Pearson et al. (1987) classified over 2000 isolates of *M. phaseolina* from maize from 13 states in the USA as chlorate-resistant and those from soybean as chlorate-sensitive. Whereas Zazzerni and Tosi (1989) on the basis of chlorate utilization concluded that there was no evidence for host specificity within *M. phaseolina,* which was also supported by Mihail and Taylor (1995) who tested 114 *M. phaseolina* isolates from different host species, soils and continents. This indicated clearly that *M. phaseolina*, despite being a phenotypically highly variable species, cannot be partitioned into distinct subspecies based on pathogenicity, pycnidium production and chlorate utilization.

Su et al. (2001), using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD), examined *M. phaseolina* isolates from soybean, corn, sorghum, and cotton root tissue and soil from fields cropped continuously with these species for 15 years. They observed no variation among isolates in restriction patterns of DNA fragments amplified by PCR covering the internal transcribed spacer (ITS) region, 5.8S rRNA and part of 25S rRNA, suggesting that *M. phaseolina* constitutes a single species and that isolates from one host species are genetically similar but distinct from those from other hosts. Using 22 primers and the banding patterns in the RAPD tests with 55 Brazilian isolates, unweighted pair-group arithmetic average (UPGMA) cluster analysis revealed three distinct genetic groups (Almeida et al. 2001). The apparent low genetic variability within each group was attributed to either lack of a sexual stage in the fungus life cycle or the easy and natural transmission of the fungus by seeds that spread the isolate throughout the Brazilian soybean-growing regions. They also noted the presence of different haplotypes in the same root. So far, none of these DNA studies has been able to differentiate isolates of *M. phaseolina* from specific hosts or geographical locations. However, Jana et al. (2003) developed a single RAPD primer OPA-13 that can be used to differentiate numerous isolates of *M. phaseolina* from soybean, sesame, groundnut, chickpea, cotton, common bean, okra and 13 other hosts. Saleh et al. (2010), on the basis of RFLP data and the sequence of the rDNA-ITS region, divided 143 isolates into four clusters: one containing most of the isolates from maize and soybean, two others containing isolates from wild plants and sorghum, and a fourth containing a single isolate recovered from *Solidago canadensis* in the tallgrass prairie. In India, RAPD data based on 14 random primers on 20 isolates showed 98.1% polymorphism in different isolates (Das et al. 2008). Similarly, Babu et al. (2010) using RAPD fingerprinting on 50 isolates from different host range and diverse geographical locations in India reported that the primer OPB-08 shown the maximum polymorphism and the UPGMA clustering separated 50 isolates into ten groups at more than 65% similarity level. In their study, these groups correlated well with the geographical locations with exceptions for isolates obtained from Eastern and Western Ghats. But the presence of monomorphic bands in all isolates indicated that all isolates might have evolved from a common ancestor but due to geographical isolation followed by natural selection and genetic drift might have segregated into subpopulations.

As such, the matter of host specialization is still debatable, and as such, systematic studies are required to reveal the factual position.

Survival and Recurrence

Survival

The fungus survives in soil and host crop debris generally through microsclerotia (Short et al. 1980). Normally, after harvest of the infected crop, the microsclerotia are protected in the fallen crop residues and are then released into the soil after crop residues break down. Ramakrishnan (1955) thought it reasonable to assume that the fungus does not exist in free soils, but inside vegetable debris, which it colonizes, and remains there either as mycelium, or in some resting stage. The fungus could also survive as mycelium in asymptomatic seeds and microsclerotia in symptomatic seeds (Hartman et al. 1999).

Longevity and overwintering of sclerotia

Microsclerotia which are distributed generally in clusters in the soil are confining mainly at a depth of 0–20 cm (Mihail 1989) and persist within the soil up to 3 years under adverse conditions such as low soil nutrient levels and temperatures above 30°C (Dhingra and Sinclair 1977). Depending on environmental conditions and association of the microsclerotia with the host residue, microsclerotia can normally survive for 2–15 years, (Short et al. 1980; Baird et al. 2003). The fungus is also known to survive for up to 3 years as mycelium in asymptomatic seeds or as microsclerotia in symptomatic seeds (Hartman et al. 1999). Therefore, understanding the importance of soybean host debris, seed and soil as an inoculum source has special significance in disease management strategies.

Role of soil environment, planting and other factors

The germination of microsclerotia in soybean fields is favoured by dry soils, high soil C : N ratios of amendments, low bulk density (Dhingra and Sinclair 1975; Gangopadhyay et al. 1982) and oxygen concentrations $>16\%$ (Todd et al. 1987). These factors influence the oxygen diffusion rate and nutrient balance in the soil (Saxena 1980; Craig 1992) and may encourage the germination of resting spores of soil-borne pathogens. In soybean fields, microsclerotia size, density and total populations were related to the number of years of planting maize and soybean (Short et al. 1978).

A small, but significant decrease in inoculum density was found with succeeding years. Within the infected host, microsclerotia become detectable in large numbers when the tissues began to decay. Infected soybean stubble appears to be one of the major sources of inoculum of *M. phaseolina* for seedling infection in the spring.

The number of the microsclerotia of *M. phaseolina* in soil was directly related to the severity of charcoal rot, which in turn was inversely related to soybean yield, while no consistent correlation between the severity of host infection and charcoal rot incidence was found (Short et al. 1980). Percentage infection increased linearly with an increase in the inoculum density of microsclerotia in the soil (Sheikh and Ghaffar 1979). They observed that with an increase in air and soil temperature, the residual *M. phaseolina* population re-initiates growth resulting in the formation of a significant number of detectable propagules. The nature of these propagules is not fully understood, but the propagules are involved in the formation of new microsclerotia. The number of cells per sclerotium was directly related to the size of microsclerotia, which was dependent on available nutrients of the substrate on which the propagules were produced. On culture media, large microsclerotia produced more germ tubes than small ones, which were sensitive to soil fungistasis, but in the spermosphere of soybean, microsclerotia germinated within 2–3 mm of the seed surface and produced 1–7 germ tubes/germinated microsclerotia.

Laboratory Cultural Characteristics

Acimovic (1964) observed that the most rapid mycelial growth and rapid production of microsclerotia were on PDA and on agar with onion and oats. Microsclerotia were smallest in size at 30°C and largest at 15 and 35°C. According to Waseer et al. (1990), the pathogen grew best on PDA or potato dextrose juice at 35°C; little growth occurred at either 20 or 40°C.

Characteristically, grey to black colonies of pathogen develop on the medium, depending on the latter's nutrient status. Aerial mycelia with complete or partial growth may or may not develop on media. Some isolates show concentric growth. Branching of the mycelium normally occurs at acute angle but hyphal branch from parent hyphae generally arise at right angles (Hartman et al. 1999). On PDA, Waseer et al. (1990) observed abundant microsclerotial production with colourless hyphae, which turn light brown in old age. These microsclerotia are smooth or irregular in shape, black in colour and rich in oils (Dhingra and Sinclair 1978). Pycnidia are not produced except under specific incubation conditions (Gaetán et al. 2006). When produced, they are black and globose (100–200 μ in diameter) and contain single-celled fusiform conidia (Dhingra and Sinclair 1978). Goth and Ostazeski (1965) observed production of pycnidium in culture obtained on propylene oxide-sterilized leaf tissue. Similarly, pycnidium are also produced on both groundnut meal irradiated with UV light and on filter paper treated with vegetable oil on peptone or asparagines agar (Knox-Davies 1966).

Both from host tissues and from soil, *M. phaseolina* can easily and readily be cultured in a semi-selective medium of PDA amended with chloroneb and streptomycin sulphate. Using selective medium, Papavizas and Klag (1974) developed a technique for direct isolation and estimation of inoculum density of *M. phaseolina* from soil. The average recovery of microsclerotia from artificially infested soils was 80%. Average numbers of microsclerotia in naturally infested soils vary considerably from 0 to 1000/g soil. Recently, a double-antibody sandwich enzyme-linked immunosorbent assay was employed for specific detection and quantification of *M. phaseolina* in plant tissues (Fouda et al. 2009).

Seed Pathological Aspects

Macrophomina phaseolina was found to be associated with seeds of rice, wheat, blackgram, greengram and soybean when tested for seed-borne fungi (Agarwal et al. 1972). Seed-borne infection of *M. phaseolina* can be detected by blotter paper, agar plate and modified Potato-Sucrose-Agar [PSA + Penta Chloro Nitro Benzene (PCNB)] methods (Kushi and Khare 1978).The blotter method was found better than the agar plate method. The three incubation conditions, namely 20°C black light fluorescent tubes, 28°C near ultraviolet and 28°C artificial day light proved to be equally effective. Pretreatment with NaClO in the agar plate method reduced the percentage incidence of some pathogens including *M. phaseolina*.

At Jabalpur, M.P., India, soybean seeds harvested from the first three planting dates in rainy season 1971 and stored in a refrigerator (1–5°C) had greater frequency of internally borne fungi including *M. phaseolina* and a lower germination than those stored at room temperature at 15–45°C (Nicholson and Sinclair 1973). Infected seeds have indefinite black spots and blemishes on the seed coat and reduced germination (Gangopadhyay et al. 1970), and thus the pathogen can be carried on and in the seed coat and is capable of infecting the radicle (Dhingra and Sinclair 1978). Gangopadhyay et al. (1973) observed *M. phaseolina* in cotyledons and seed coats from artificially infected seeds, which were plated separately on PDA following surface sterilization. Kunwar et al. (1986) reported that microsclerotia developed on roots, hypocotyls and cotyledons during incubation of soybean seeds infected with *M. phaseolina*. Gangopadhyay et al. (1970) recovered the fungus from the radicles of a few seedlings. Seed infection of soybean with *M. phaseolina* at levels of 1.5–8.0% has been reported (Michail et al. 1979). Kunwar et al. (1986) recovered the pathogen from all seeds showing symptoms of infection and also from 8 out of 1000 asymptomatic seeds harvested from naturally infected plants from Illinois. The fungus produced microsclerotia in 4% of asymptomatic seeds after 2 day of incubation at 25 ± 2°C on acidified (pH 4.5) PDA. It was also observed that the

microsclerotia developed near or adjacent to the seed coat, endosperm and hypodermis. The fungus, apparently, could penetrate and colonize soybean seeds without producing symptoms, but subsequently formed microsclerotia in asymptomatic seeds when conditions were favourable for seed germination. Kunwar et al. (1986) observed that microsclerotia were formed in the cotyledons of asymptomatic seeds after 3–4 day of incubation and in the hypocotyle – radicle axis, after 4–5 day. Kumar and Singh (2000) reported that the fungus was invariably present in the seed coat of all the infected seeds and moved into the cotyledons (including embryonal axis) of the 40% infected seeds. The pathogen remained viable for 15 months in seeds at room temperature and was transmitted to seedlings during germination by local contact.

Histological examination of symptomatic seeds showed that hyphae and microsclerotia were ecto- and endophytic, while hyphae were inter- and intracellular in tissues of the seed coat, endosperm and embryo. During germination of the asymptomatic seeds, the inoculum could invade the cotyledons and embryo with in 3–5 day, and microsclerotia developed on such seeds.

Biology, Ecology, Disease Development and Life Cycle

Individual microsclerotium comprises of 50–200 or more individual cells united by a septal pore in each cell and can germinate repeatedly during the crop-growing season. The microsclerotia are variable in size (50–150 μm), depending on the nutrients availability in the substrate on which the propagules are produced (Short and Wyllie 1978). *Macrophomina phaseolina* sometimes also produces pycnidia in host crops (Mihail and Taylor 1995), but their importance in the epidemiology of the fungus depends on the host species involved as well as on the fungal isolate (Ahmed and Ahmed 1969). The pycnidiospores in *Macrophomina* are ellipsoid to obovoid and measure 20–24 $\times$ 7–9 μm.

Growth of the fungus in soil is fueled by nutrients stored in the microsclerotia, so growth continues even after the soil nutrient levels become insufficient for fungal competitors to grow. On the account of this characteristic, the pathogen competes well with other soil pathogens when soil nutrient levels are low and the temperature is above 30°C. Microsclerotia germinate from a few cells at a time on the surface of, or in close proximity of the roots. Root exudates induce germination of microsclerotia, and when in proximity to or on the roots, microsclerotia germinate from a few cells at a time. Numerous germ tubes are formed, which give rise to appresoria on the anticlinal walls of epidermal cells. The appresoria penetrate the epidermal cell walls by mechanical pressure and enzymatic digestion or via wounds and natural openings (Hartman et al. 1999). Ammon et al. (1975) observed that within 3 day of inoculation, appressoria are produced on the root surface at the tip of the primary hyphae. During the initial stages of pathogenesis, the mycelium penetrates the root epidermis of mostly 7–42-day-old soybean plants and is primarily restricted to the intercellular spaces of the cortex of the primary roots. Consequently, adjacent cells collapse and heavily infected plantlets may die. At flower onset stage, the infected plants show necrotic lesions on stems, branches and peduncles. From pod peduncles, the fungus spreads to the pods and invades grains. Heavily infected plants die prematurely probably due to the production of fungal toxins, e.g., phaseolinone or botryodiplodin (Ramezani et al. 2007) and plugging host vessels. In soybean, formation of microsclerotia is triggered by flowering and pod setting (Wyllie and Calvert 1969) and may be indicative of initiation of death of the host (Short and Wyllie 1978). Disease results in poor seed-setting in pod and reduces seed size, which finally lead to yield loss. After plant death, colonization by mycelia and formation of microsclerotia in host tissue continue until the tissues have dried. After decay of root and plant debris, microsclerotia are released into the soil and the cycle continues.

Short et al. (1978) developed a technique for determining mycelial and microsclerotial propagative units in infected soybean tissues for the measurement of disease development. Following plant death, microsclerotia were most numerous in the roots, less abundant in the lower half of the stem and least in the upper half. They suggested that differential propagule enumeration in diseased tissues quantitatively measures the degree of compatibility between selected soybean cultivars and *M. phaseolina* (Short and Wyllie 1976). Bressano et al. (2010) described a new method to infect intact soybean plants with *M. phaseolina* and characterized initial penetration of the infection process. A general limitation when studying the early events in the infection process of a soil-borne plant pathogen is the detection of the hypha arrival at the root surface. *Macrophomina phaseolina* produces hyaline structures that are undetected by staining methods based on fungal chitin dyes and were identified exclusively by stained lipid vesicles produced by the pathogen within host tissues. They suggested that this method may be applicable to examine prepenetration and the penetration phases of other soil-borne fungi as well as the early responses of the host plant.

Climate and Other Factors Influencing Infection, Symptom Expression and Severity

A high level of root infection can occur before reproductive development if there is a preponderance of hot and dry weather early in the growing season (Olaya and Abawi 1993). Visible symptoms of the disease in the field are most apparent under conditions that reduce plant vigour, e.g., poor soil fertility, high seeding rates (Sinclair and Backman 1989), low soil water content (Kendig et al. 2000), high temperatures (Mihail 1989) and root injury (Bowman et al. 1986). The appearance of disease is also related to rainfall pattern but air temperature is the most critical factor. The timing of host reproduction is another factor that

has a strong influence on charcoal rot development. Colonization of the pathogen was higher when plants were subjected to water stress and postflowering water stress resulted in greater intensity of charcoal rot (Tosi and Zazzerini 1990; Diourte et al. 1995). It was also observed that the population density of *M. phaseolina* increased slowly from the V5 to R6 growth stages and then rapidly from the R6 to R7 growth stages (Mengistu et al. 2011). Under the humid tropical conditions of south-western Nigeria, high soil moisture levels were unfavourable for the growth and pathogenicity of *M. phaseolina*, while low soil moisture levels favoured these fungal traits (Wokocha 2000). Similarly, in cowpea, *M. phaseolina* population in the soil was negatively correlated with soil moisture and positively correlated with maximum soil temperature (Gupta and Gupta 1986). Reduction in the number of viable microsclerotia may be brought approximately in the field by keeping soil moisture above 60% of its moisture-holding capacity at 30°C or above for 3–4 weeks (Dhingra and Sinclair 1974). The disease index of charcoal rot disease was significantly low (2.0) when 14-day-old soybean cultivar Samsoy 1 seedlings inoculated with *M. phaseolina* were watered regularly to maintain a high (60–70%) soil moisture level. On the contrary, the disease index was high (5.0) when inoculated seedlings were water-stressed and grown under low (10–20%) soil moisture levels (Tosi and Zazzerini 1990). However, water management can limit, but not prevent the colonization of *M. phaseolina* (Kendig et al. 2000). Dry conditions favour survival of microsclerotia in the soil, but mycelial growth and infection require moist conditions and are favoured by a temperature above 27°C (Hagedorn 1991).

Controlled environment experiments have shown that the maximum infection occurs in inoculated seedlings grown at 30–40°C and temperature above 45°C reduced disease incidence (Meyer et al. 1974). Mihail (1989) observed a marked increase in mortality when soil temperature at 5-cm depth reached 28–30°C. In India, the epiphytotics occur in soybean-growing areas where temperatures range from 35 to 40°C.

Production of microsclerotia, as well as severity of the charcoal rot, is also known to be influenced by cultivar (Ndiaye 2007) in cowpea.

The stage of development at which a plant is most likely to exhibit symptoms of *M. phaseolina* infection varies to some extent with host species. Legume crops tend to be more susceptible at the seedling stage than cereal crops. There are reports of availability of *M. phaseolina* infection at the early seedling stage when grown in autoclaved soil (Gangopadhyay et al. 1970). However, the distinction again needs to be made between the optimum time for infection and the main period of symptom development, which are often temporally separated. In soybean, plant maturity is found to be the only factor affecting microsclerotial production, which is independent of moisture stress and temperature. The aetiological link of *M. phaseolina* with seedling infection and symptom expression in the mature plant is not fully understood. It seems that the pathogen invades the base of the stem and then remains dormant until the plant becomes predisposed to infection after the onset of flowering. Kirkpatrick et al. (2006) reported the recovery of *M. phaseolina* from soybean significantly lower from plants flooded at the V4 growth stage when compared with the non-flood treatment. Wrather et al. (2008) found that drought tolerance of the soybean genotypes and colonization by *M. phaseolina* were not related, and hence, they suggested that additional research is required to determine whether the effects of drought and infection by *M. phaseolina* are additive, synergistic or independent.

Overwintering and Longevity of Pathogen in Roots and in Soil

Baird et al. (2003) reported that the fungus *M. phaseolina* isolation frequencies declined linearly over time, which may have been affected by root segment degradation or interactions with other microorganism. Pathogenic and saprophytic fungi are major components of the soil micro-flora and are known to over-winter on crop debris. The pathogen over-winters primarily as microsclerotia in soybean root and stems tissues and continues to survive even after the root and stem tissues decay (Short et al. 1980). Furthermore, the longevity of the microsclerotia of *M. phaseolina* obtained from other hosts was reported to be several years (Dhingra and Sinclair 1977). They also reported that the formation of microsclerotia is an important survival mechanism and thus influences longevity of *M. phaseolina*. Microsclerotia continue to germinate and infect host tissues during subsequent growing seasons unless destroyed by environmental factors or other microorganisms.

Baird et al. (2003) reported that the survival frequency of *M. phaseolina* was greater at the 0 cm depth (14.0%) than at 7.6 cm (8.8%) and at 25.4 cm depth (9.8%). Plant tissue degradation by microorganisms could have been partially responsible for the reduced longevity of *M. phaseolina* at the 7.6 and 25.4 cm depths. Occurrence of *Trichoderma* spp. population is higher with increased burial depth of 7.6 and 25.4 cm compared with the surface debris and may lower the survival frequency of *M. phaseolina* with increased depth. Farming practices, which enhance shredding of the debris and burial through tillage practices, may increase the rate of wood degradation or colonization by saprophytic fungi that reduce the relative longevity of *M. phaseolina* in debris. The cellulose-degrading fungi such as *Trichoderma* spp. and basidiomycetes may be more active within the soil due to available moisture and more stable temperatures resulting in greater activity.

Herbicide stress is known to have no or minor influence (Cerkauskas et al. 1982; Bowman et al. 1986; Mengistu et al. 2009) on longevity of microsclerotia and its effect depends on the type of herbicide, particularly on soybean plants with root injuries (Canaday

et al. 1986). In a field study made by Canaday et al. (1986), root colonization by *M. phaseolina* was significantly increased by stresses induced with chloramben and 2,4-DB, while it was significantly reduced with stress induced by alachlor. Glyphosate and vernolate had no effect. Trifluralin significantly reduced root colonization by *M. phaseolina* in the absence of significant herbicide stress. This indicated that root colonization is more closely related to root injury than to herbicide stress.

The mycelium of *M. phaseolina* can also retain infectivity for substantial time period. Mycelia originating from microsclerotia of two strains of *M. phaseolina, after transfer to ampules and storage in liquid N,* retained their original level of pathogenicity over a period of 9 months, upon inoculation into the hypocotyls of 2-week-old soybean seedlings (Wyllie and Fry 1973).

Mutual Relationship with Other Microorganisms

A synergistic relationship of *M. phaseolina* has been found with *Endogyne calospora* (Schenck and Kinloch 1974), *Heterodera glycines* on soybean (Short et al. 1980) and *Meloidogyne incognita* on several hosts (Sharma et al. 1980; Short et al. 1980; Mishra et al. 1988). In white clover, *M. phaseolina* also tends to be associated with higher final densities of the plant-pathogenic nematodes *Meloidogyne trifoliophila, Helicotylenchus dihystera* and *Heterodera trifolii* (Zahid et al. 2002). In contrast, in a pot experiment, the simultaneous addition of *M. phsaeolina* and *Meloidogyne javanica* resulted in reduced numbers of nematode galls in mungbean. This was attributed to the effect of toxic metabolites, produced by the fungus, on the nematode (Gupta and Mehta 1989). Sharma and Khan (2010) observed a delay of 8 day in the life cycle of *Meloidogyne javanica* in the presence of *M. phsaeolina* in balsum. Increased incidence of *M. phaseolina* was observed when it was co-inoculated with nematodes, which may be due to increased root invasion or enhanced water stress by nematode, making the host more susceptible to *Macrophomina*. The lesion nematode *Pratylenchus zeae* and *M. phaseolina* act synergistically to reduce plant growth in sorghum (Bee-Rodriguez and Ayala 1977). Norton (1958) reported similar results with *Pratylenchus hexincisus*. *Macrophomina phaseolina* has a poor competitive saprophytic ability and has the capability to make changes in soil fungistasis. Reduction in bacterial populations has been correlated with increased microsclerotial germination, while increased microbial activity can decrease microsclerotial viability (Filho and Dhingra 1980). *Rhizobium* strains indigenous to Pakistan were reported to inhibit growth of *M. phaseolina* in culture (Zaki and Ghaffar 1987). Arbuscular mycorrhizal (AM) fungi and *Rhizobium* spp. can promote plant growth and control plant fungal diseases (Akhtar et al. 2011). Integration of AM fungus with *Rhizobium* sp. appears to be a promising approach for sustainable agriculture. Arbuscular mycorrhizal fungi increase soil nutrients and water absorption, while root-nodule bacteria fix atmospheric nitrogen and produce antibiotics and phytoalexins (Akhtar et al. 2011). *Aspergillus flavus*, a common soil-inhibiting fungus, has also been reported to decrease infection by *M. phaseolina* in peanut kernels (Jackson 1965).

Control Measures

Cultural practices

With the help of crop management, population levels can be lowered and soil moisture can also be retained, which in turn can culminate in a reduced incidence of charcoal rot as well as in the reduction of microsclerotia. Rotation with non-host crops for 2–3 years is considered necessary to lower *M. phaseolina* infection levels in severely infested fields (Ndiaye 2007). It was also suggested that 1-year crop rotation with any of the crops among maize, sorghum and cotton is a means of maintaining microsclerotial populations at an acceptable level (15 microsclerotia per gram of soil) with regard to soybean crop (Todd et al. 1987).

Limited information is available on the direct role of fertility or plant nutrition in charcoal rot management in soybean (Todd et al. 1987). Increase in nitrogen:phosphorus:potassium (NPK) supply (Csondes et al. 2008) is important for charcoal rot management in soybean.

Soil population of the pathogen was greater in soybean fields under no-tillage (NT) than either disc or mould broad tillage (Wrather et al. 1998) as tillage practices influence the distribution and size of plant debris left on the soil surface, which in turn influences the diversity and density of pathogenic and saprophytic fungi (Baird et al. 1993). In contrast, Mengistu et al. (2009) demonstrated that the NT system had fewer CFU of *M. phaseolina* in root and stem tissues of soybean than the conventional tillage system indicating that NT may provide a less conducive environment to support the *M. phaseolina* population Infact, data of Wrather et al. (1998) were based on soil samples taken only at planting and did not include population density from plant tissue and from soil during harvest.

Through irrigation, the infection of *M. phaseolina* can be reduced in several crops such as soybean (Michail et al. 1979) and *Phaseolus vulgaris* (Diaz-Franco and Cortinas-Escobar 1988). Irrigation combined with seed treatment with *Trichoderma viride* at the time of moisture stress could reduce the intensity of disease to approximately 50% (Ansari 2010). Soybeans can escape disease if full-season maturity groups are planted to avoid the hottest and driest condition during the postflowering period (Bowen and Schapaugh 1989).

Charcoal rot disease can be controlled by organic amendments such as farmyard manure, neem and mustard cake (Rathore 2000). Lodha et al. (2002) observed 63–72% reduction in *M. phaseolina* -induced plant mortality at harvest in clustebean by soil amendment with pearl millet compost. Ndiaye (2007) obtained good control of charcoal rot and substantial increase

in cowpea yield by amending field with 6 metric tonnes of compost/ha.

Muthusamy and Mariappan (1992) observed that neem or gingelly cake at 3%, inactivated 90 and 80% microsclerotia from soybean, respectively, by stimulating germination and lysis as compared to the untreated control. They observed maximum germ tube production and lysis in coconut cake extracts. Solarization (Katan et al. 1980) has been suggested as a possible method for managing soil-borne pathogens, but alone was not effective in controlling *M. phaseolina* in field soils (Mihail and Alcorn 1984). Ndiaye (2007) concluded from experiments that under conditions where solarization alone does not provide sufficient control, the combination with organic amendments improves yields and reduces infection by *M. phaseolina*.

Integration of cultural practices can provide effective disease management. Such practices include crop rotation and manipulation in date of sowing (Bristow and Wyllie 1975), application of lime, fertilizer, deep ploughing and amendments with organic matters (Collins et al. 1991), applications of organic manures and micronutrients coupled with irrigations in case of sunflower and sorghum, etc., rotations, soil fertility, sowing density and irrigation (Ploper et al. 2001), soil application of Zn along with *Bradyrhizobium japonicum* and *T. viride* (Ansari 2010).

Chemical control

Soil treatment

In order of decending efficacy, benomyl, thiophanate-methyl, thiram, thiobendazole, triforine and captan when mixed with soil decreased the viability of microsclerotia in soil, and in soybean stem pieces in laboratory tests (Ilyas et al. 1976). In field studies with inoculated soil, fungicides did not affect emergence, but microsclerotia numbers were greatly reduced by benomyl and to a lesser extent by the other fungicides. Seedling infection was controlled best by benomyl and thiobendazole but both showed some phytotoxicity (Ilyas et al. 1975).

Soil fumigation with sodium methyldithiocarbamate reduced populations of the pathogen on soybean residue and in the roots of plants grown in field plots (Kittle and Gray 1982). Fumigation with methyl bromide significantly increased soybean yields and reduced the number of viable microsclerotia and prevalence of *M. phaseoli* (Watanabe et al. 1970).

Seed treatment

Seed treatment by fungicides is effective to some extent in reducing losses caused by *M. phaseolina* in crops, which are particularly vulnerable at the seedling stage. Vir et al. (1972) reported that, thiophanate-methyl and furcarbanil (each at 1000 ppm) provided the best degree of control against *M. phaseolina* on soybean. Seed treatment with carbendazim 50 WP (2.0 g / kg seed) and thiophanate-methyl (1.0 g/kg seed) was effective in eliminating the pathogen *M. phaseolina* from infected seeds of soybean (Kumar and Singh 2000). Gupta and Chauhan (2005) also recommended seed treatment with captan at 3 g/kg or thiram at 3 g/kg or thiram + carbendazim (2 : 1) at 3 g/kg or thiram + carboxin at 2 g/kg seed. Seed treatment with captafol and mancozeb was effective in control of root rot and soybean seedling emergence (Singh et al. 1990).

Growth regulators and hormones

Gibberellic acid (GA) and 2,3,5-triiodobenzoic acid (TIBA) reduced the severity of charcoal rot in soybean, while indole-3-acetic acid and kinetin gave inconsistent responses (Oswald and Wyllie 1973). The effect of TIBA was attributed to a change in the pattern of vessel elements that impeded fungal colonization and not to fungal toxicity (Kroll and Moore 1981). In other reports (Chakraborty and Purkayastha 1981), foliar sprays of GA reduced and of kinetin increased the disease severity. *In vitro* tests showed that both the hormones increased the growth of the pathogen. Soybean grown in the growth chamber and in the field was treated with indole-3-acetic acid and kinetin, prior to inoculation with *M. phaseolina*. either increased, did not alter, or decreased disease severity, depending on the applied concentration and growth conditions, while gibberellins and triiodobenzoic acid limited disease severity under all experimental conditions (Oswald and Wyllie 1973; Chakraborty and Purkayastha 1981).

In vitro *efficacy of fungicides*

Growth rate of *M. phaseolina* (*Rhizoctonia bataticola*) on Potato Dextrose Agar medium containing 5, 10 or 50 μg/ml of fungicides BD 18654 and topsin M (thiophanate-methyl) was significantly less than the respective controls (Kirkpatrick and Sinclair 1973). Dubey (1989) isolated *M. phaseolina* from diseased soybean and cultured on Czapex Dox medium individually amended with1000 μg/ml of each tetracycline, ampicillin, grisofulvin and agrimycin 100, which inhibited fungal growth by 73.9, 50.9, 40.7 and 13.0%, respectively. The fungicides Bavistin 50 WP (carbendazim at 5000 μg/g), Dithane M-45 75 WP (mancozeb at 10 000 μg/g) and PCNB 75 WP (quintozene at 10 000 μg/g) were effective in reducing the survival of *M. phaseolina* than the herbicides Fernoxone 80 WP (2,4-D), Benthiocarb 50 EC (thiobencarb) and Machete 50 EC (butachlor) or the insecticides namely BHC 50 WP (HCH), endocel 35 EC (endosulfan) and Nuvacron 36 EC (monocrotophos), each at 10 000 μg/g concentration on 100% active ingredient basis. High pesticide concentration (5000–10 000 μg/g) was required to kill the inoculum from precolonized soybean stem pieces in soil (Dubey 1991). Using PCNB, Anahosur et al. (1983) reported 87% inhibition of *M. phaseolina* growth *in vitro*. At 30 ppm and above, the growth of the pathogen was completely inhibited by azadirachtin and carbendazim, whereas mancozeb inhibited the growth by 87.3%. A gradual decline in the viability of microsclerotia was recorded with an increase in

incubation time. All the microsclerotia lost their viability after 96 h of treatment with azadirachtin, mancozeb and carbendazim (Dubey and Kumar 2003).

Biological control

Farm practices which increase residue destruction immediately after harvest or those that enhance *Trichoderma* spp. populations may directly or indirectly lower the relative longevity of soil-borne pathogens, including *M. phaseolina* (Baird et al. 2003). In India, use of *T. viride* or *Trichoderma harzianum* as a seed treatment (4–5 g/kg seed) has been recommended for the management of charcoal rot in soybean (Gupta and Chauhan 2005). Seed treatment with *Pseudomonas aeruginosa* reduced infection of *M. phaseolina* by 14–100%, depending on the strain of bacterium and the variety of soybean used (Ehteshamul-Haque et al. 2007). Hashem (2004) evaluated the efficacy of three antagonists (*T. harzianum, Epicoccum nigrum* and *Paecilomyces lilacinus*) and found that *Epicoccum nigrum* and *Paecilomyces lilacinus* suppressed the growth of *M. phaseolina* in soybean by producing an inhibition zone, whereas *T. harzianum* suppressed the growth by overgrowing the *M. phaseolina*. In India, the inoculation of seeds or roots with *Rhizobium japonicum* reduced the severity of charcoal rot disease in soybean on account of the fungitoxic action of rhizobitoxine (Chakraborty and Purkayastha 1984; Pearson et al. 1984). Rhizobitoxine was also recovered from the roots inoculated with *R. japonicum* (Chakraborty and Purkayastha 1984). In a field study in Egypt, incidence of root rot and wilt caused by *M. phaseolina* was reduced by 30.5–87.5% compared with the control, using plant growth-promoting rhizobacteria (PGPR) (*Azotobacter chroococcum*, *Azospirillum brasilense*, *Bacillus megaterium* var. *phosphaticum*, *Bacillus cereus* and *Pseudomonas fluorescens*) alone or in combination (El-Barougy et al. 2009). *Bacillus subtilis* was also reported to inhibit the growth of *M. phaseolina in vitro* condition (Dawar et al. 2010). Choudhary (2011) characterized plant growth-promotion activities of Rhizobacteria A5F and FPT721 and *Pseudomonas* sp. strain GRP3 for their antagonistic activities against *M. phaseolina*. Plant growth-promoting rhizobacteria can inhibit pathogens via the production of hydrogen cyanide and/or fungal cell wall-degrading enzymes, e.g., chitinase and ß-1,3-glucanase (Hayat et al. 2010).

Several antagonistic, rhizosphere-inhibiting fungi and bacterial endophytes of soybean were also identified in *in vitro* tests (Senthilkumar et al. 2009). Pratt (2006) observed that when ground poultry litter was added to soil with microsclerotia at 5% by weight, their survival was significantly reduced, and when litter was added at 10%, survival of the microsclerotia was nearly eliminated. Based on these results, he emphasized that animal waste and other agricultural by-products may be evaluated for biocontrol activity against sclerotia of *M. phaseoliona* in soil.

Botanicals

Some plants have shown to exert high antimicrobial/antifungal activities. This might be due to their chemical constituent which have higher water solubility and diffusion coefficient and also due to their higher hydrogen bonding potential (Bisht et al. 2010).

The botanicals found as effective as chemical fungicides against *M phaseolina* were as follows: (i) essential oil actinidine isolated from *Nepeta clarkei* (Saxena and Mathela 1996), (ii) neem oil (Alice et al. 1996), (iii) aqueous extract of Cymbogon citratus (Bankole and Adebanjo 1995), (vi) powder of *Datura fastulosa* (Ehteshamul-Haque et al. 1992) and (v) dry hot water extract of *Cleome viscoasa* and *Mentha longifolia* and dry methanol extract of *Berberis aristata*, *Conyza bonariensis*, *Cleome viscoas*a, *Lantana camera* and *Vitax negunda* (Arora and Kaushik 2003). Seed infection of *M. phaseolina* was completely inhibited by dipping the seeds for 5 min in ginger, garlic and neem extracts (Hossain et al. 1999).

Host resistance

Plants recognize invading pathogens and respond biochemically to prevent invasion or inhibit colonization in plant cells. The soybean phytoalexin glyceollin inhibited the growth of *M. phaseolina*, *Diaporthe phaseolorum* var. *meridionales*, *Phytophthora sojae*, *S. sclerotiorum*, *Cercospora sojina*, *Phialophora gregata* and *Rhizoctonia solani* (Lygin et al. 2010). Other studies suggest the possible involvement of peroxidase and polyphenoloxidase system (Gangopadhyay and Wyllie 1974), glyceollin (Chakraborty and Purkayastha 1987) and antigenic substances (Chakraborty and Purkayastha 1983) in susceptibility or resistance to the disease. Host resistance may be the best alternative for disease control because of reduced cost and eco-friendly to manage the disease (Bristow and Wyllie 1984; Bowen and Schapaugh 1989; Smith and Carvil 1997; Smith and Wyllie 1999). Short et al. (1978) reviewed the role of mycelial and microsclerotial units within soybean tissues in providing resistance to disease.

Searches for specific resistance to charcoal rot in soybean have been unsuccessful (Schneider et al. 1974), attributed in part to the variability of the pathogen (Todd et al. 1987). Variation in root tissue colonization by *M. phaseolina* among soybean genotypes was observed by many researchers (Bristow and Wyllie 1984; Pearson et al. 1984; Smith and Carvil 1997; Kendig et al. 2000; Paris et al. 2006). Weimer (1947) found that the cultivars Roanoke, Volslate, FG 30261-1 and Woods Yellow of soybean showed tolerance to charcoal rot. Gangopadhyay et al. (1973) reported that the soybean cultivar Hill was least susceptible and Harosoy most susceptible to the infection. Pearson et al. (1984) reported soybean cultivars Bay, Essex, Forrest and Sprite had the slowest rates of *M. phaseolina* colonization. Few soybean genotypes, including 'Delta and Pineland 3478', 'Hamilton', 'Jackson II', 'Davis' and 'Asgrow 3715,' have been identified as either moderately resistant or tolerant under field conditions (Smith

and Carvil 1997; Smith and Wyllie 1999). They also recommended the cultivation of cultivars that do not have a late reproductive stage that coincides with periods of drought stress.

To evaluate soybean genotypes, Mengistu et al. (2007) proposed a classification system based on a colony-forming unit index (CFUI) and identified four soybean genotypes out of 24 genotypes as moderately resistant to *M. phaseolina* in USA. Wrather et al. (2008) also found variation in root tissue colonization among soybean genotypes. At the R6 growth stage, root tissue colonization was significantly lower for PI 416937 (2640 cfu/g root) and N987288 (1525 cfu/g root) than Hutcheson (4000 cfu/g root). Later on, Mengistu et al. (2011) suggested growth stage R7 should be taken as the optimum stage for assessing disease using CFU to evaluate soybean genotypes. In India, soybean varieties, namely NRC 2, NRC 37, JS 71 05, LSb 1 and MACS 13 with low susceptibility to charcoal rot, have been identified and recommended (Gupta and Chauhan 2005).

Identification of molecular marker(s) linked to the charcoal rot resistance gene would greatly facilitate screening of breeding materials and thus accelerate the development of new resistant cultivars. For this purpose, a core set of 100 diverse soybean genotypes were subjected to screening for resistance by Talukdar et al. (2009) in India. In their study, none of the genotypes were immune but seven genotypes (viz. DS 9712, DS 9814, JS 335, PK 564, EC 439618, EC 439619 and DS 61) were resistant. Parental polymorphism and purity of the F_1 hybrids was established using simple sequence repeats (SSR) markers. It has also been observed that the expression of the disease reaction is continuous, that is, it started from highly susceptible through moderately resistant to highly resistant. It might indicate the involvement of more than one locus in controlling the resistance of the disease. Advancement has been made to develop mapping population to map QTL for charcoal rot resistance in soybean and identification of linked molecular markers is in progress (Talukdar et al. 2009).

Wani et al. (2010) reviwed the role of RNA interference (RNAi) in the development of disease-resistant varieties and further suggested that such goal can be achieved in a more selective and robust manner with the success of genetic engineering techniques. In this regard, RNAi has emerged as a powerful tool to overcome the threats posed by viruses, fungi and bacteria. The application of tissue-specific or inducible gene silencing in combination with the use of appropriate promoters to silence several genes simultaneously will result in the protection of crops against destructive pathogens. RNAi application has resulted in successful control of many economically important diseases in plants. This molecular technique can be utilized to protect soybean from charcoal rot.

Conclusion

Charcoal rot is primarily a root and lower stem disease, but may extend into the upper stem tissue as well. While seedlings may become infected, charcoal rot is considered to be a disease of older plants occurring mid-season. The disease is more severe when plants are under stress from moisture or nutrients, excessive plant densities, soil compaction, improperly applied pesticides, nematodes or other pathogens.

There are also some reports that indicated that the disease could be reduced by the applications of organic manures and micronutrients coupled with irrigations in the case of sunflower and sorghum. Charcoal rot of soybean can also be controlled by integrating rotations, soil fertility, sowing density and irrigation. *Macrophomina phaseolina* is a polyphagous and unspecialized pathogen and studies conducted so far, did not reveal clear-cut evidence of host specialization.

Although good progress in research on charcoal rot in soybean has been made during the past decade, still systematic studies are required to bridge the gap in knowledge of physiological variability and pathogenicity, which are to be characterized genetically using polymorphism analysis of the ITS region and other relevant molecular markers-related techniques, so that, higher levels of disease resistance can be achieved in order to obtain the stable yield and quality seeds. Selection of effective crops for crop rotation, use of composting along with biocontrol agents and/or fungal antagonists and AM fungi, soil solarization or other organic amendments could be the other areas of research, which can hold a high promise for the containment of pathogen as well as disease in the absence of highly resistant varieties and very effective chemicals. Simultaneously, research work is to be strengthened to develop high yielding and durable charcoal rot-resistant varieties. To achieve this goal, a better understanding of the life cycle of the fungus, its interaction with the soybean plant at ecological, genetic and physiological level, that is, regulation of the expression of disease-related genes is considered fundamental issues for the soybean improvement.

Possibilities may be explored to utilize RNA-mediated gene silencing technology like RNAi by supreesing the specific gene(s) governing susceptibility to charcoal rot in soybean for successful management of the disease.

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