PATHOGENIC AND GENETIC DIVERSITY IN POPULATIONS OF *Sclerospora graminicola*, THE INCITANT OF DOWNY MILDEW IN PEARL MILLET

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M. Sc. (Ag.)

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CERTIFICATE

Mrs. B. PUSHPAVATHI has satisfactorily prosecuted the course of research and that the thesis entitled **"PATHOGENIC AND GENETIC DIVERSITY IN POPULATIONS OF *Sclerospora graminicola*, THE INCITANT OF DOWNY MILDEW IN PEARL MILLET"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

K. Chandrasekhara Rao 21.7.03

Date : 21, July, 2003
Place : Hyderabad

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Chairman of the Advisory Committee

CERTIFICATE

This is to certify that the thesis entitled **"PATHOGENIC AND GENETIC DIVERSITY IN POPULATIONS OF *Sclerospora graminicola*, THE INCITANT OF DOWNY MILDEW IN PEARL MILLET"** submitted in partial fulfilment of the requirements for the degree of **"DOCTOR OF PHILOSOPHY IN AGRICULTURE"** of the Acharya N.G. Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Mrs. B. PUSHPAVATHI** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of the investigation has been duly acknowledged by the author of the thesis.

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Date : 21 July, 2003

(B. PUSHPAVATHI)

DECLARATION

I, **B. PUSHPAVATHI** hereby declare that the thesis entitled **"PATHOGENIC AND GENETIC DIVERSITY IN POPULATIONS OF *Sclerospora graminicola*, THE INCITANT OF DOWNY MILDEW IN PEARL MILLET"** submitted to ACHARYA N. G. RANGA AGRICULTURAL UNIVERSITY for the degree of **"DOCTOR OF PHILOSOPHY IN AGRICULTURE"** is the result of original research work done by me. I also declare that the material contained in the thesis has not been published earlier.

Date: 21st July, 2003
Place: Hyderabad

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ABSTRACT

Studies on pathogenic and genetic diversity, mating types, and inheritance of virulence in *Sclerospora graminicola* (Sacc.) Schroet., the pearl millet downy mildew pathogen were undertaken at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. A total of 21 isolates from major pearl millet growing areas of India were selected from the collections maintained at ICRISAT in the form of oosporic inocula. From these oosporic inocula, asexual inocula were generated on a universally susceptible host genotype, 7042S and were denoted as parental isolates.

During the establishment of parental isolates, the isolate, Sg 153 recorded the highest disease incidence (76.15%) with shortest latent period (6.00 days) and the isolate Sg 021 (1.97%) and Sg 004 (2.61%) recorded the lowest disease incidence with longest latent period (30.00 days). Isolates collected during 1997 recorded significantly higher disease incidence than those collected in the previous year.

The 21 parental isolates were evaluated for pathogenicity on a set of ten host differentials and were found highly variable for virulence, disease incidence, disease reaction, latent period, virulence index and oospore production potential. Among the parental isolates, the isolate Sg 139 was found highly virulent and Sg 110 the weakly virulent. Based on disease incidence, the parental isolates were classified into seven pathotype groups. A representative isolate from each group was identified and ten single-zoospore isolates (SZIs) from each representative isolate were established for further studies.

Considerable variation was found among the SZIs of Sg 139 and Sg 110 for virulence, disease incidence, disease reaction, latent period and virulence index on host differentials used. Among the SZIs of Sg 139, the isolate Sg 139-4 was found highly virulent while, the isolate of Sg 139-1 was the least virulent. In case of SZIs of Sg 110, the isolate Sg 110-3 was found highly virulent and the least virulent was Sg 110-9.

A high level of polymorphism was detected among the parental isolates using AFLP analysis with three primer combinations. Presence or absence of few unique bands was observed in isolates Sg 004, Sg 025, Sg 026, Sg 139 and Sg 115. Based on similarity index, the isolates were classified into eight groups. The cluster composition varied for AFLP analysis and virulence analysis, and these two were found independent.

Results of mating type study demonstrated the existence of two mating type groups designated as Mat A and Mat B. Of the 70 SZIs evaluated, 62 were found self-sterile and 8 self-fertile, indicating the predominant heterothallic nature of the fungus with rare occurrence of homothallism. Among the 70 SZIs, the overall frequency of both the mating types was approximately equal.

The inheritance of virulence in isolates of *S. graminicola* was studied by hybridizing the isolates of Sg 139-4 (Mat A) and Sg 110-9 (Mat B), which differed extremely in their virulence on a host differential IP 18292. Observations in F_1, F_2 and backcross generations indicated the dominant nature of avirulence over virulence, and the role of single gene pair in governing the virulence in isolates Sg 139-4 and Sg 110-9, and resistance in IP 18292. The pattern of segregation of virulence on IP 18292 also suggested the presence of gene-for-gene interaction between *S. graminicola* and *Pennisetum glaucum* (L) R. Br.

LIST OF SYMBOLS AND ABBREVIATIONS

%	=	per cent
@	=	at the rate of
µl	=	microliter
ATP	=	Adenosine triphosphate
C	=	Celsius
CD	=	Critical Difference
cm	=	Centimeter
dNTP	=	deoxynucleotide 5' – triphosphate
EDTA	=	Ethylene Diamine Tetra Acetic acid
et.al	=	and others
Fig.	=	Figure
g	=	gram
ha	=	hectare
mg	=	milligram
ml	=	milliliter
mM	=	millimolar
rpm	=	revolutions per minute
S.Ed.	=	Standard error at degrees of freedom
SDS	=	Sodium Dodecyl Sulphate
Sg	=	*Sclerospora graminicila*
Tris	=	tris-hydroxymethyl amino methane

Introduction

CHAPTER-I

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the important cereal crops in the arid and semi-arid tropics of the world, particularly in the Indian subcontinent and the Sahelian zone of West Africa. The crop is grown annually on 37.40 m ha with an annual production of 29.21 m tons in the world (FAO, 2002). India and Africa together produce more than 90 per cent of the world output (Yadav, 1996). In India, it is the fifth most important cereal after rice, wheat, maize and sorghum, occupying an area of 9.55 m ha and having an annual production of 8.35 m tons (AICPMIP, 2003).

The crop is grown on sandy marginal soils and under harsh climatic conditions where no other crop can successfully be grown. Although the crop is quite hardy, it still suffers from various biotic and abiotic stresses. One of the major biotic yield-reducing factors is the disease downy mildew, caused by *Sclerospora graminicola* (Sacc.) Schroet. The disease is of significant economic importance in India and elsewhere.

The downy mildew disease was reported for the first time on pearl millet in India during 1907 (Butler, 1907). Since then, it remained as a disease of minor importance until 1970. With the introduction of high yielding hybrids (HB 1 and HB 3) during late 1960s in India, the disease appeared in an epidemic form in 1971 in certain parts of India (Safeeulla, 1977; Singh *et al.*, 1993). Subsequent to this epidemic, several resistant cultivars have succumbed to downy mildew after being widely cultivated by farmers and have been withdrawn from cultivation (Singh, 1995; Thakur *et al.*, 1998a). During the last

three decades, considerable progress has been made in the areas of pathogen biology and disease epidemiology, and in developing management practices, particularly host-plant resistance (Singh *et al.*, 1997). However, the re-occurrence of downy mildew in severe form during 1993-96 on few F_1 commercial hybrids in some farmers' fields in Maharashtra (Thakur *et al.*, 1999) shows that the disease will continue to be a major limiting factor to the exploitation of the high yield potential of improved cultivars, particularly single cross F_1 hybrids.

The pathogen *S graminicola* is an obligate biotroph, which reproduces asexually by means of sporangia that liberate motile zoospores, and sexually through oospores. The fungus is mostly heterothallic but homothallism may also exist (Michelmore *et al.*, 1982). These characteristics of the fungus make it highly variable like its host pearl millet, which is a highly outcrossing crop species. Sexual reproduction also provides new genetic recombinations resulting in evolution of pathogen populations with greater virulence and parasitic fitness. Under these circumstances, utilization of host-plant resistance is the only feasible way to manage the disease. Therefore, a thorough understanding of the mechanisms underlying the evolution of pathogenic variation in *S graminicola* and resistance operating in pearl millet genotypes are highly essential to develop cultivars with stable and durable resistance.

The present investigation was therefore undertaken with the following objectives :

1. Characterization of isolates of *S graminicola* for pathogenic variability
2. Characterization of isolates *S. graminicola* for genetic diversity
3. Identification of mating types among *S graminicola* isolates
4. Determination of inheritance of virulence in *S. graminicola*

Review of Literature

CHAPTER - II

REVIEW OF LITERATURE

A brief review of literature relevant to the objectives for the present investigation is presented under the following titles :

2.1 General

2.2 Pathogenic variability in *Sclerospora graminicola*

2.3 Molecular markers for genetic diversity

2.4 Sexual compatibility in downy mildews

2.5 Inheritance of virulence

2.1 GENERAL

2.1.1 Pearl millet - the host

Pearl millet (*Pennisetum glaucum*) is an important coarse grain cereal and forage crop of the arid and semi-arid tropics of the Indian subcontinent and several African regions (Khairwal *et al.*, 1999). The crop is thought to be originated in Sahelian zone of West Africa and subsequently introduced into India (Purseglove, 1976). As a semi-arid crop, it is traditionally a component of the dryland cropping system. It can withstand high temperatures and severe soil- moisture stress, and recover rapidly to exploit periods of more favourable conditions (Andrews *et al.*, 1985; Bidinger *et al.*, 1987). No other crop has been found equal to or surpassing the dependability of pearl millet as a source of food for a large number of subsistence farmers who inhabit the semi-arid tropics of Asia and Africa (Cummings, 1975).

Pearl millet is mainly grown for grain and forage on about 15 m ha in Africa and 10 million ha in Asia (De Wit, 1986). In India, it is the fifth most important cereal food crop and is chiefly grown in the states of Rajasthan, Maharashtra, Gujarat and Haryana (Govila, 1994). It has also been recognised as a valuable forage crop, because of its robust and quick growth with high fodder yield, in the South-eastern USA and dry areas of Australia (Yadav, 1996).

Pearl millet is a naturally outbreeding crop species. For breeders, it is an excellent species for genetic research, because of its low chromosome number (2n=14), short life cycle (80-90 days), high multiplication ratio (upto 1:1000), ratooning ability and the ease with which cross pollination can be done due to its protogyny flowering nature (Govila, 1994).

Before the 1970s, pearl millet local cultivars were predominantly grown. Although those cultivars produced some yield under adverse conditions, the average yields were low. During this period, some sporadic efforts were made to improve these cultivars through simple selection methods, but they had limited spread and thus little impact on production (Yadav, 1996).

The discovery of cytoplasmic male-sterile (CMS) lines (Burton, 1958) heralded the beginning of a successful breeding programme in India. A CMS line Tift 23A, bred at Tifton, Georgia (Burton, 1965), proved to be the most successful seed parent with 75-100 per cent increase in yield over the local Indian cultivars (Kumar *et al.*, 1983). It was, however, extensively utilised in India and five hybrids (HB 1 to HB 5) based on this line were released for

commercial cultivation from 1965 to 1969 (Dave, 1987). Production increased from 3.5 to 8.0 million tons. However, intensive cultivation of hybrids based on a single male-sterile (MS) line resulted into cytoplasmic and genetic homogeneity leading to a downy mildew epidemic in 1971, and pearl millet grain production fell to 3.3 million tons. Thereafter, a series of downy mildew resistant hybrids (HB 1, HB 3, BJ 104 and MBH 110) released from time to time have succumbed to downy mildew within 5-7 years of their cultivation (Singh *et al.*, 1997). This was due, primarily, to the lack of genetic diversity and inadequate resistance to downy mildew in MS lines rather than to cytoplasmic susceptibility (Yadav *et al.*, 1993). However, much greater efforts are now being made to breed for downy mildew resistance and the MS lines currently being used are highly resistant (Rai and Singh, 1987; Thakur *et al.*, 2001). Consequently, the life span of single-cross hybrids produced in recent years is longer than for those produced in the 1970s (Yadav, 1996).

2.1.2 Downy mildew - the disease

2.1.2.1 Geographical distribution

Pearl millet downy mildew is widely distributed in the temperate and tropical areas of the world and is especially widespread in India (Nene and Singh, 1976) and Africa (N'Doye *et al.*, 1986; Chevaugeon, 1952; Saccas, 1954; Bouriquet, 1963). Saffeeulla (1976) reported that *Sclerospora graminicola* had been recorded in more than 20 countries. According to Jeger *et al.* (1998), it was reported from 51 countries of the world, which include the continents Asia, Africa, Europe and America. In India, the pathogen is present in all the states where pearl millet is cultivated (Singh *et al.*, 1993)

and appear consistently in the states of Maharashtra, Rajasthan and Gujarat with high disease incidence (Thakur *et al.*, 2001).

2.1.2.2 Economic importance

The magnitude of grain yield reduction largely depends on disease severity levels and the stage of crop growth during infection. Total loss may occur in plants exhibiting downy mildew infection in seedling stage and green-ear infection during earhead formation (Thakur, 1987). The pathogen, *Sclerospora graminicola*, has been reported to cause severe yield loss in many regions of the world (Williams, 1984). In Africa, pearl millet downy mildew incidence is frequently between 0 and 50 per cent, although crop loss is normally in the range of 0-20 per cent (ICRISAT, 1985; Frison and Sadio, 1987; Labe *et al.*, 1987; Werder and Manzo, 1992; Mbwaga *et al.*, 1993; Ouendeba *et al.*, 1995). During a survey in Niger, the incidence of pearl millet downy mildew ranged from 0.8 per cent to over 40 per cent (Gilijamse *et al.*, 1997).

In some reports, the grain losses upto 60 per cent have been reported from various African countries (DeCarvalho, 1949; Doggett, 1970; King and Webster, 1970). Nene and Singh (1975) reported loss estimates of 6 per cent in East China, 45 per cent in India, 60 per cent in Mozambique and 30 per cent in high yielding hybrids in India. In one locality in Israel, the disease caused an almost complete loss of a 70 ha crop grown for fodder (Kenneth, 1966).

It is also reported that the worldwide annual pearl millet grain yield losses due to downy mildew do not exceed 20 per cent (Khairwal *et al.*, 1999). However, this disease can assume an alarming level when a single genetically

uniform pearl millet cultivar is repeatedly and extensively grown in a region where the pathogen is present (Andrews, 1987; Singh *et al.*, 1987a). This was clearly demonstrated in HB 3, a popular hybrid in India in the early seventies, when pearl millet grain production was reduced from 8.2 million t in 1970-71 to 3.3 million t in 1971-72 (AICMIP, 1972). This reduction was to a large extent, due to downy mildew epidemic, in which yields in some fields were reduced by 60-70 per cent. Subsequent to this epidemic, grain yield losses continued to occur quite frequently due to downy mildew epidemics in India (Singh *et al.*, 1987b). Thakur *et al.* (1999) during their surveys recorded a disease incidence of 80 to 100 per cent on a few hybrids in states of Maharashtra, Rajasthan and Gujarat in India, accounting for a considerable yield loss.

Mayee and Siraskar (1982) found a significant correlation (r = 0.99) between the disease incidence and yield loss in pearl millet. It was estimated that a disease incidence of 60-80 per cent resulted in grain yield loss of about 30-40 per cent. Translated into economic terms the loss realised by a farmer was substantial, in the tune of Rs. 1500-2000 (US$ 1 = Rs. 42) per ha (Mayee and Siraskar, 1982). Clearly, downy mildew is the obvious culprit preventing the realisation of increased production of pearl millet in the state, despite cultivation of several F_1 hybrids with high yield potential.

2.1.2.3 Symptomatology

The disease is mainly characterised by two types of symptoms *viz.*, downy mildew and green ear. Leaf symptoms begin as chlorosis at the base of the leaf lamina, and successively younger leaves show a progression of

greater coverage of leaf area by symptoms. Under conditions of high relative humidity (> 95 %) and moderate temperature (20-25°C), the affected leaf portions support a massive asexual sporulation, generally on the abaxial surfaces, giving them a downy appearance. Severely infected plants are generally stunted and do not produce panicles (Singh, 1995). Green ear symptoms become visible at panicle emergence and appear as transformed floral parts into leafy structures, which can be total or partial on panicles. These leafy structures are chlorotic, and sometimes produce spores. In latent infections, green ear is the only manifestation of the disease (Singh *et al.*, 1997).

2.2 PATHOGENIC VARIABILITY IN *Sclerospora graminicola*

Variation in plant pathogenic fungi arise largely through sexual recombination, heterozygocity and somatic recombination, mutation and hybridization (Singh, 1986). Large shifts in pathogenicity occurs due to changes in host cultivar and environment. In general, highly variable populations are better adapted than those with little variation. Pathogenicity or virulence has been used as the genetic marker in all the studies where variability has been assessed through virulence surveys, using host differentials having different resistance genes (Wolfe and Knott, 1892).

Sclerospora graminicola is known for its highly variable nature. The variation in pathogenicity can evolve either due to the environmental and varietal differences or the selection pressure exerted by a host genotype.

2.2.1 Variability due to environmental and varietal differences

The first evidence of pathogenic variation in *S. graminicola* based on pearl millet cultivars was reported in 1973 where HB 3 was found resistant at

Mysore, but susceptible at some other locations in India (Bhat, 1973). Nene and Singh (1976) interpreted this variation as being due to existence of races in the pathogen. Several promising pearl millet genotypes which evaluated in international multilocational downy mildew nurseries showed environmental variation in their downy mildew incidence during 1976 - 1977 (ICRISAT, 1980).

Variation in pathogenicity of *S. graminicola* populations from different locations in Africa and India has been demonstrated by several researchers (Ball, 1983; Ball and Pike, 1983; Ball and Pike, 1984; Ball *et al.*, 1986). Pathogen collections from different geographic regions differ in pathogenicity when tested on a set of host cultivars. Populations from sub-sahelian regions of West Africa were more pathogenic than the Indian ones on Indian cultivars (Ball and Pike, 1984). Similarly, populations form Burkina Faso, Nigeria and Niger were generally more aggressive than those from Senegal, Zambia or India, but no differences in aggressiveness were found between Indian and Zambian populations (Ball *et al.*, 1986). In India, variations in pathogenicity of *S. graminicola* populations were reported from Mysore and Gulbarga on the pearl millet cultivar HB 3 (Shetty and Ahmed, 1981); from Patancheru on MBH 110 and NHB 3 (ICRISAT, 1989) and from Patancheru and Durgapura on NHB 3 (Singh and Singh, 1987).

Werder and Ball (1992) studied ten lines of pearl millet and inoculum from four different sources in West Africa, and confirmed variability both in the reaction of different host lines and the virulence of different pathogen isolates.

In all the above studies, only the percentage seedling infection was used to measure host-pathogen interactions. Therefore, variation in virulence and aggressiveness was not clearly distinguished. Keeping this in view, Thakur and Shetty (1993) reported the interactions of 15 single-oospore isolates with a set of pearl millet genotypes for various components of aggressiveness : latent period, infection efficiency and sporulation rate. They also found considerable variation among the isolates tested. Later, the existence of variation in pathogenicity of single-zoospore isolates of the fungus was also demonstrated in the pearl millet downy mildew system (Thakur and Shetty, 1993).

Thakur *et al.* (1997) evaluated 61 pearl millet genotypes against six pathotypes and observed highly significant effects of host genotypes, pathotypes and their interaction on incidence and latent period.

Recently, a highly significant variability in downy mildew incidence across the 46 genetically diverse male sterile lines was reported by Thakur *et al.* (2001). The study implied that the variability was due to genetic divergence among the lines, the pathotypes and their interaction (Thakur *et al.*, 2001).

2.2.2 Variability due to host-directed selection

The emergence of a new pathotype in an asexual population is not solely an outcome of genetic recombination, but could be the effect of host genotype - directed selection for specific virulence in the pathogen population. Thakur *et al.* (1992) observed the response of host cultivar directed selection on virulence in a population of *S. graminicola*. In their study, at ICRISAT, Patancheru, the collection of pathogen from NHB 3 or 7042S, which was less

virulent on MBH 110 and 852 B was passed through several asexual generations on MBH 110 and 852 B. Within 12 generations of selection on MBH 110 and 5 generations of selection on 852 B, highly host-specific virulences, comparable to their respective field pathotypes, were identified. These results indicate that genetic variation for host genotype specific virulence exists within field populations of the pathogen, and that selection through asexual generations can rapidly increase the quantitative virulence of the population to the specific host genotypes.

Though the A_1 cytoplasm of MS lines has been shown not to be involved in susceptibility to downy mildew (Kumar *et al.*, 1983; Yadav *et al.*, 1993), the genetic uniformity of single-cross F_1 hybrids provides little or no barrier to the pathogen in rapidly adapting to the new cultivar (Talukdar *et al.*, 1999) and exerts strong selection pressure on the pathogen population for its shift to host-specific virulence. With the commercial cultivation of such hybrids, emergence of several cultivar-specific virulences have been detected (Thakur and Rao, 1997) and popular hybrids, such as HB 1, HB 3, BJ 104, BK 560 and MBH 110 have succumbed to downy mildew and these have been withdrawn from cultivation (Singh *et al.*, 1997).

The results from the International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN), as determined by disease reaction on a set of cultivars after several years of operation at diverse locations in India and West Africa clearly indicated the existence of distinct virulences in the pathogen populations and provided further evidence for the evolution of several

pathotypes within and between the countries in Asia and Africa (Thakur, 1995; IPMDMVN, 1999).

Thakur *et al.* (1998a) determined the pathological identity of a population of *S graminicola* from a pearl millet cultivar *Nokha* local from Jodhpur, Rajasthan and identified it as a new pathotype. Further studies suggested that *Nokha* pathotype was different from the previously described pathotypes of *S graminicola* and was the most virulent isolate reported to date from India. The results of field surveys and studies conducted by Thakur *et al* (1999) provided yet another evidence for the host-directed selection and confirmed the emergence of a new virulent pathotype, specific to a widely grown hybrid MLBH 104, which caused substantial damage to the crop in Maharashtra during the 1993-96 crop seasons. Thus, the downy mildew pathogen has evolved rapidly to keep pace with the changing cultivars in India (Thakur *et al.*, 2001).

2.3 MOLECULAR MARKERS FOR GENETIC DIVERSITY

Genetic studies in any organism requires precise and easily scoreable heritable characters or markers. Fungi are often microscopic and have few phenotypic markers, such as vegetative compatibility, mating types or specific virulence (Leslie, 1993; McDonald and McDermot, 1993; Michelmore and Hulbert, 1987). Over the past three decades, techniques have been developed for the analysis of variants of specific enzymes in animal and plant tissues (Tanksley and Orton, 1983). Proteins in crude extracts are electrophoretically separated on starch or polyacrylamide gels and the gel is stained to visualise the zones containing the specific enzyme activity. Sometimes, the genetic

interpretation of enzymes that exhibit many bands, such as phosphatases and esterases, may be more difficult. However, isozyme polymorphisms have provided useful markers for genetic studies in several fungi (Tooley *et al.*, 1985; Tooley *et al.*, 1989; Spielman *et al.*, 1990; Linde *et al.*, 1990; Burdon and Roberts, 1995).

The advent of highly versatile, molecular markers, which are based on differences in DNA sequences has made it possible to conduct basic studies on population and evolutionary biology in fungi. These techniques include DNA hybridization methods, such as endogenous genomic and mitochondrial Restriction Fragment Length Polymorphisms (RFLP), DNA fingerprinting and PCR-based Randomly Amplified Polymorphic DNA (RAPD) markers. These techniques have been widely used to estimate the genetic diversity in several fungal species of zygomycetes, ascomycetes and deuteromycetes (Weising *et al.*, 1995).

RFLP probes and RAPD markers have been successfully used to estimate the genetic diversity in many fungal pathogens (Anderson and Pryor, 1992; Milgroom *et al.*, 1992; Guthrie *et al.*, 1992; Levy *et al.*, 1991). Though, only the morphological, virulence and mating type markers were most commonly used in *Phytophthora* and some downy mildew fungi; RFLP and RAPD polymorphisms were used for genetic characterisation of *Bremia lactucae* Regel, the lettuce downy mildew pathogen (Hulbert *et al.*, 1988; Hulbert and Michelmore, 1988) and *Plasmopara halstedii*, the sunflower downy mildew pathogen (Vick *et al.*, 1990). In *Magnaporthe grisea*, the rice blast pathogen, genomic repetitive DNA sequences were also used to detect

polymorphisms (Hammer *et al.*, 1989). A RAPD analysis exhibited genetic dissimilarities among the isolates of *Colletotrichum sublineolum*, the causal agent of sorghum anthracnose (Thakur *et al.*, 1998c).

Genetic variation within and between populations of *S. graminicola* showing different virulence phenotypes were studied using RAPD technique (Zahid, 1997). The relatedness of four populations of *S. graminicola*, from Africa and India were assessed using 65 arbitrary oligonucleotide primers and all the four populations were found highly variable. Polymorphism was also observed among 20 isolates of *S. graminicola* from different geographic locations using 34 primers. The dendrogram plotted for relatedness among the isolates revealed two distinct clusters of which one indicated African isolates and the other indicated Indian isolates (Zahid, 1997).

RFLP of mitochondrial DNA (mtDNA) has also detected polymorphisms in a number of phytopathogenic fungi, such as *Fusarium oxysporum* f.sp. *melonis* Snyder & Hansen (Jacobson and Gordon, 1990), *Pythium* (Martin and Kistler, 1990) and *Phytophthora* (Forster *et al.*, 1990; Forster and Coffey, 1991; Stammler *et al.*, 1993; Lacourt *et al.*, 1994). High levels of diversity were evident in *Phytophthora citricola* Sawada and *P. capsici* Leonian Distinct subgroups could also be distinguished in *P. citrophthora* (Sm. & Sm.) Leon. whereas mitochondrial RFLP patterns were very uniform in *P. palmivora* Butler (Forster *et al.*, 1990). Some intraspecific variability was detected using mitochondrial DNA RFLP among 87 isolates of *P. parasitica* Dast. collected world wide (Lacourt *et al.*, 1994).

Hypervariable markers, such as mini- and micro-satellite markers are also known to show high level of DNA sequence variation (Jeffreys *et al.*, 1985). The polymorphisms arise from the variation in the number of repeat units present in tandem arrays in the fungal genome. The human minisatellite probes 33.6 and 33.15 have been successfully used to distinguish pathotypes of *C. gloeosporioides* (Penz.) Sacc. (Braithwaite and Manners, 1989). Commercially, many probes such as minisatellites, M13, PV47 or simple repetitive oligonucleotides, $(CA)_8$, $(CT)_8$, $(CAC)_5$, $(GTG)_5$, $(GACA)_4$ and $(GATA)_4$ have also been used in DNA fingerprinting. DNA fingerprinting using oligonucleotides, such as $(GATA)_4$, $(GTG)_5$, $(CA)_8$ and $(TCC)_5$ has been reported to detect variation among isolates of *Ascochyta rabiei* (Weising *et al.*, 1991). In case of filamentous fungi, such as *Pencillium, Aspergillus* and *Trichoderma*, oligonucleotide probes such as $(GATA)_4$ along with M13 minisatellite probe, have revealed informative DNA fingerprinting patterns (Meyer *et al.*, 1991). Similarly, microsatellites $(GAA)_6$, $(GACA)_4$ and $(GATA)_4$ showed high levels of DNA polymorphism among the pathotype of *S. graminicola*, the pearl millet downy mildew pathogen (Sastry *et al.*, 1995) and four races of *Fusarium oxysporum* f.sp. *ciceri* (Padwick) Snyd. & Hans. (Barve *et al.*, 2001).

Of all the molecular methods, the more recent and advanced fingerprinting method is Amplified Fragment Length Polymorphism (AFLP), which combines the reliability of RFLP with the power of the PCR technique (Vos *et al.*, 1995). Though various molecular methods have been used to detect and quantify genetic variation in fungi and other plant pathogens,

AFLP has been used very effectively to detect genetic variation in several plant pathogenic fungi (Majer *et al.*, 1996; Wang *et al.*, 1998).

Pongam *et al.* (1999) detected genetic variation among the isolates of *Leptosphaeria maculans* (Desmaz.) Ces. & De Not using AFLP analysis. The genetic variability in 36 isolates of *Fusarium udum* Butler (Sivaramakrishnan *et al.*, 2002a) and 43 isolates of *F. oxysporum* f.sp. *ciceri* (Sivaramakrishnan *et al.*, 2002b) from different locations in India was also assessed using RAPD and AFLP techniques. Though the two molecular markers detected high levels of polymorphism among the pathogen isolates, the AFLP technique proved better in assessing the genetic diversity among the isolates than RAPDs. The AFLP technique was also used to develop a genetic linkage map for *P. infestans*, a plant pathogenic fungus of the class Oomycetes (Vanderlee *et al.*, 1997).

2.4 SEXUAL COMPATIBILITY IN DOWNY MILDEWS

Unlike many fungi, downy mildews are diploid for the majority of their life cycle in which sexual reproduction involves fertilization of an oogonium by passage of nuclear material from an antheridium, leading to the formation of an oospore (Michelmore *et al.*, 1988). Oospores are thick-walled survival structures of the fungus and act as primary source of infection (Singh *et al.*, 1993). It is essential to know in detail about the nature and sexuality of the pathogen to understand the mechanisms underlying its pathogenic variability. Unfortunately, the inconsistency of oospore germination (Michelmore and Ingram, 1981) and the erratic rate of recovery of sexual progeny have been often a limitation to the studies on sexual reproduction (Shaw, 1983).

Therefore, at present, studies on this aspect in downy mildews are few and limited.

The existence of sexual reproduction in *B. lactucae* was a matter of controversy until it was clearly demonstrated by Humphreys-Jones (1971) and confirmed by Tommerup *et al.* (1974). In these studies, and those of Ingram *et al.* (1975) and Fletcher (1976), oospores were produced in lettuce plants unpredictably and in low numbers. However, it was shown in further studies that *B. lactucae* is capable of regular and predictable production of large number of oospores in lettuce tissues. Many isolates which are incapable of sexual reproduction when cultured alone, produced oospores in large numbers when cultured in combination with certain other isolates. This demonstrated the existence of heterothallism in *B. lactucae*. In a survey of 39 isolates only two compatibility types were identified and were designated as B_1 and B_2. The survey did not reveal any other compatibility types (Michelmore and Ingram, 1980).

Michelmore *et al.* (1982) made a systematic study on sexual system of *S. graminicola*. The results illustrated the heterothallic nature of the fungus. The isolates studied could be assigned to one of two sexual compatibility types that have been designated G_1 and G_2. Scanning electron micrographs showed hyphae of two morphological types, similar to those observed preceding the formation of gametangia in the heterothallic *B. lactucae*. They explained the patterns of asexual and sexual sporulation of *S. graminicola* in terms of differential colonization of the apices of young host plants. In this study, a few oospores were found infrequently in plants infected with one of

the isolates. It could be due to a form of self-fertility or due to the isolate being a mixture of the two compatibility type isolates, with one at a low frequency. Self-fertility due to secondary homothallism has also been observed in *B. lactucae* (Michelmore and Ingram, 1982) and in several predominantly heterothallic *Phytophthora* species (Mortimer *et al.*, 1978). The low level of self-fertility in *S. graminicola* may also be a form of secondary homothallism as it is similar to that observed with self-fertile isolates of *B. lactucae*.

Inter and intra-continental sexual compatibility in *S. graminicola* has been reported by Idris and Ball (1984). Oospore collections obtained from diverse locations in West Africa and India were tested for sexual compatibility, alone and in every possible combination. Oospores were produced with some combinations of isolates but not in others indicating the presence of two compatibility types, G_1 and G_2. These were found in approximately equal proportions. Tests for cross-compatibility were made by combining isolates of opposite sexual compatibility types from Africa and India. Isolates were cross compatible not only within continents but also between continents. This study also provided an evidence for existence of secondary homothallism in *S. graminicola*.

While working with genetics of virulence in Californian populations of *B. lactucae* Ilott *et al.* (1989) found strong correlation between sexual compatibility type and pathotype. Virulence phenotypes and sexual compatibility types were determined for 116 Californian populations of *B. lactucae*, collected between 1982 and 1986. All the isolates were grouped into

one of the three distinct pathotypes on the basis of their virulence phenotypes. All pathotype I isolates had the B_1 sexual compatibility type, and all pathotypes II and III isolates had the B_2 sexual compatibility type.

To identify the compatible mating types among the isolates of *S. graminicola* Rao *et al.* (1994) inoculated the seedlings of 7042S with six isolates (PT 1 through PT 6) of *S. graminicola* singly and in all possible combinations. They observed abundant oospores in a paired inoculation with the isolates PT 2 and PT 3 and designated them as Mat 1 and Mat 2, respectively. They further evaluated a total of two hundred and one isolates of *S. graminicola* collected from various pearl millet genotypes at ICRISAT, Patancheru and reported the occurrence of both homohtallism and heterothallism in *S. graminicola.*

Heterothallic behaviour has also been reported for other Oomycetes fungi such as *Peronospora effusa* (Inaba and Morinaka, 1984), *P. parasitica* (Kluczewski and Lucas, 1983; Sheriff and Lucas, 1989) and *Phytophthora infestans* (Mont.) de Bary (Gallegly and Galindo, 1958; Shaw *et al.*, 1985; Malcolmson, 1985; Tantius *et al.*, 1986) on their respective hosts.

2.5 INHERITANCE OF VIRULENCE

Studies on inheritance of virulence in *S. graminicola* are lacking. Hence, the more relevant studies in other well studied downy mildew fungus, *B. lactucae* and few other fungi are reviewed hereunder.

Early studies on the inheritance of virulence were carried out in the early 1930s with the fungus *Ustilago* (Nicolaisen, 1934) and the study

indicated that the capacity to incite a host and the expression of either a susceptible or resistant disease reaction was under Mendalian control. Flor (1955) proposed the gene-for-gene hypothesis as the simplest explanation of the results of studies on inheritance of pathogenicity in the flax rust fungus, *Melampsora lini*. On the varieties of flax that had one gene for resistance to the parent race, F_2 cultures of the fungus segregated into monofactorial ratios. On varieties which had 2, 3 or 4 genes for resistance to the parent race, the F_2 cultures segregated into bi- tri-, or tetra factorial ratio (Flor, 1947). This suggested that for each gene that conditioned resistant reaction in the host there was a corresponding gene in the pathogen that conditioned pathogenicity. Each gene in either member of a host-pathogen system may be identified only by its counterpart in the other member of the system. This relationship implies that the pathogenicity genotype of a rust culture can be established by a study of its selfed cultures on differential lines with single genes conditioning rust resistance (Flor, 1965). This implication made possible the study of hetrozygosity of races from the natural *M lini* population to determine usefulness of host genes. These studies provided knowledge of which genes for resistance would be most effective in a breeding program and how genes in *M lini* are inherited (Flor, 1965; Statler, 1979). Virulence has been conditioned by single recessive genes in most inheritance studies of *M lini* but digenic recessive combinations have been reported (Statler and Zimmer, 1976; Statler, 1979).

Person and Sidhu (1971) reviewed the literature on the genetics of pathogenicity and generalized that the virulence/avirulence was usually under

Mendelian control. They also conducted a study on virulence of *Ustilago hordei* (Pers.) Lagerh. isolates and reported that the virulence was recessive and governed by single gene. Such monogenic inheritance of virulence was also observed by Lim *et al.* (1974) in case of *Drechslera turcica* isolates.

Blanch (1980) made crosses between isolates of *Gaeumannomyces graminis* var. *tritici* differing in pathogenicity and reported that the pathogenicity was under multiple gene control.

A hybrid between two biotypes of *Ustilago nuda* (Jens.) Rostr. produced segregating progenies that were used to identify two genes for virulence on five cultivars of barley. Gene Unv_1 was responsible for virulence on Warrior, Compana and Valkie, while gene Unv_2 was responsible for virulence on Keystone and Bonanza. The two genes were recessive and inherited independently from each other (Thomas, 1982).

In case of lettuce downy mildew, at least 11 specific resistance factors conferring resistance in lettuce to *B. lactucae* were identified and the extensive genetic studies revealed that many of these resistance factors were inherited as dominant alleles at single loci (Crute and Johnson, 1976; Johnson *et al.*, 1977, 1978; Norwood and Crute, 1980). Complementary studies on the inheritance of virulence in the pathogen, however, were not possible until *B. lactucae* was shown to exhibit heterothallism (Michelmore and Ingram, 1980), which allowed controlled crosses between isolates of characterized virulence phenotype. Preliminary investigations with limited number of F_1 isolates form several crosses indicated that avirulence was dominant to virulence (Michelmore and Ingram, 1981; Blok, 1981).

More detailed studies on inheritance in virulence in *B. lactucae* was conducted by hybridizing two isolates which differed in their virulence on host cultivars carrying the resistance factors R_1, R_2, R_4, R_6 and R_{11}. The results demonstrated that the virulence to match resistance factors R_1, R_2, R_4 and R_{11} each segregated as single loci with avirulence dominant to virulence and the inheritance of virulence to R_6 was complex in which the ratios obtained could not readily be interpreted in Mendelian terms (Norwood *et al.*, 1983). There seemed, therefore, to be a locus for avirulence/virulence specific and complementary to each of the host resistance factors except R_6 and this corroborated the proposal of a gene-for-gene interaction between *B. lactucae* and *L sativa* (Crute and Johnson, 1976) of the type first described by Flor (1956).

Michelmore *et al.* (1984) confirmed that the virulence in *B. lactucae* to match the specific resistance genes located in lettuce cultivars is controlled as predicted by a gene-for-gene relationship. In *B. lactucae*, it was suggested that the loci controlling virulence to R_2 and R_{11} were linked (Norwood *et al.*, 1983) and linkage was also suggested between loci controlling virulence to R_5, R_8 and R_{10} (Michelmore *et al.*, 1984). Later, Norwood and Crute (1984) provided more evidence for these linkage relationships by making crosses involving 12 heterothallic isolates of the fungus. Much of the evidence was obtained from a cross in which virulence segregated simultaneously in the F_1 generation for eight of the 11 specific resistance factors examined.

The gene-for-gene hypothesis was again confirmed in lettuce and *B. lactucae* system by making crosses between heterothallic isolates of the pathogen on differential cultivars of the host (Ilott *et al.*, 1989) and was

shown to apply to many host-parasite interactions (Person and Ebba, 1975; Layton and Kuhn, 1988; Thompson and Burdon, 1992; Silue *et al.*, 1992; Al-Kherb *et al*, 1995). However, detailed studies have often shown that the interaction between some genotypes of host and pathogen are more complex (Lawrence *et al.*, 1981) and the number of genes involved in pathogenicity always depend on the host and the pathogen interaction (Statler, 1990).

Materials and Methods

CHAPTER - III

MATERIALS AND METHODS

The present investigation was carried out at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India during 1998-2001.

3.1 EXPERIMENTAL MATERIAL

3.1.1 Pathogen isolates

More than 200 *S graminicola* isolates in the form of oosporic inoculum, collected during surveys by pathologists from major pearl millet growing areas of India and were stored under cold storage (4°C) conditions at ICRISAT, Patancheru. Of these, 21 isolates representing eight important states, where pearl millet is being cultivated over a sizeable area, were selected for the present study (Table 1; Fig. 1).

3.1.2 Host genotypes

The near isogenic lines, with identified genes for downy mildew resistance, were not available in pearl millet. Therefore, a set of eight inbred lines (IP 5272-1, IP 18296, IP 18297, P 536-2, P 1564, P 2895-3, P 3281-1 and 700481-21-8) along with one resistant genotype (IP 18292) and one susceptible genotype (7042S) were selected as host differentials, on the basis of their differential reactions reported in a previous study (Thakur *et al.*, 1997). Differential reactions of these ten host genotypes to the known six pathotypes of *S. graminicola* are presented in Table 2. Seeds of these genotypes were obtained from genetic stocks maintained by

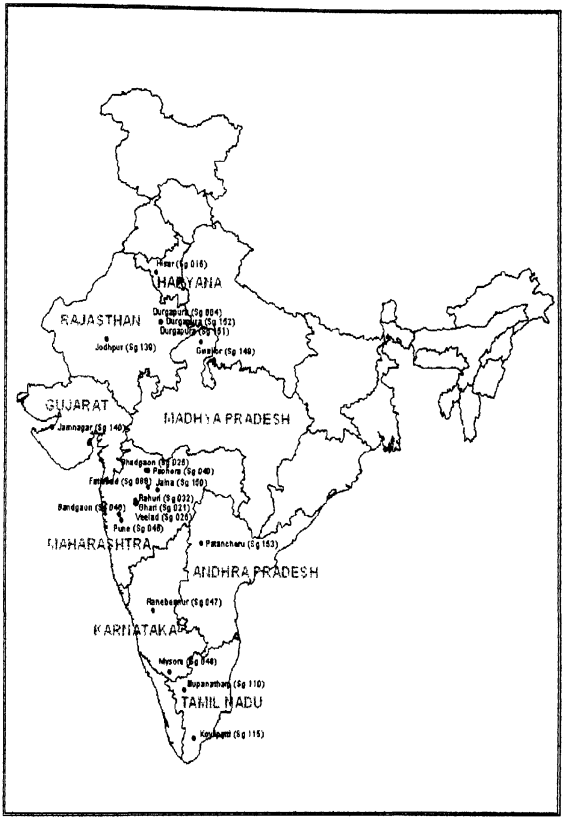

Fig.1 : Map showing the collection sites of isolates of *Sclerospora graminicola* in India where pearl millet is cultivated over a sizeable area

Table 1 : Isolates of *Sclerospora graminicola* selected for the present investigation from collections of ICRISAT, Patancheru, Andhra Pradesh, India

S.No.	Isolate designation	Source of host genotype	Year of collection	Site of collection (Location/ District/ State
1	Sg 004	7042S & HB 3	1988	ARS/ Durgapura/ Jaipur/ Rajasthan
2	Sg 015	7042S	1992	HAU/ Hisar/ Haryana
3	Sg 021	MLBH 104	1993	Ghari/ Ahmadnagar/ Maharasthra
4	Sg 025	BK 560	1993	Bhadgaon/ Jalgaon/ Maharashtra
5	Sg 026	NATH 4209	1993	Veelad/ Ahmadnagar/ Maharasthra
6	Sg 032	HB 3	1993	MPKV/ Rahuri/ Maharasthra
7	Sg 040	BK 560	1994	Pachora/ Jalgaon/ Maharasthra
8	Sg 045	MAHYCO Hybrid	1994	Pune/ Pune/ Maharasthra
9	Sg 046	EKNATH 201	1994	Bandgaon/ Pune/ Maharasthra
10	Sg 047	BK 560	1994	Ranebennur/ Dharwad/ Karnataka
11	Sg 048	7042S & HB 3	1994	Univ.of Mysore/ Mysore/ Karnataka
12	Sg 088	GK 1006	1996	Fatiabad/ Aurangabad/ Maharashtra
13	Sg 110	CO-3	1996	Illupanatham/ Kovai/ Tamilnadu
14	Sg 115	Hybrid	1996	Kovilpatti/ Tirunelveli/ Tamilnadu
15	Sg 139	Nokha Local	1997	Jodhpur/ Jodhpur/ Rajasthan
16	Sg 140	7042S/HB 3	1997	Jamnagar/ Jamnagar/ Gujarat
17	Sg 149	Local	1997	Gwalior/ Madhya Pradesh
18	Sg 150	MBH 110	1997	Mahyco Farm/ Jalna/ Maharashtra
19	Sg 151	81 A	1997	ARS/ Durgapura/ Jaipur/ Rajasthan
20	Sg 152	Local	1997	ARS/ Durgapura/ Jaipur/ Rajasthan
21	Sg 153	7042S & NHB 3	1997	ICRISAT/ Patancheru/ Medak/ A.P.

Table 2 : Differential reactions of ten selected pearl millet genotypes to the six known pathotypes of *Sclerospora graminicola*

S.No.	Pearl millet genotypes	*S. graminicola* pathotypes											
		Path 1		Path 2		Path 3		Path 4		Path 5		Path 6	
		Disease reaction	Disease incidence (%)	Disease reaction	Disease incidence (%)	Disease reaction	Disease incidence (%)	Disease reaction	Disease incidence (%)	Disease reaction	Disease incidence (%)	Disease reaction	Disease incidence (%)
1	IP 5272-1	R	4	R	3	R	0	S	87	R	1	S	56
2	IP 18296	S	29	S	18	R	0	R	0	S	14	R	2
3	IP 18297	R	0	R	0	R	0	S	39	R	0	R	0
4	P 536-2	S	13	S	19	R	1	R	0	R	2	S	38
5	P 1564	S	31	S	11	R	0	S	86	R	0	S	35
6	P 2895-3	R	0	R	1	S	12	S	50	R	0	S	15
7	P 3281-1	R	0	R	1	R	0	R	0	R	0	S	14
8	700481-21-8	S	39	S	49	S	59	R	0	S	25	S	43
9	IP 18292	R	0	R	0	R	0	R	0	R	0	R	0
10	7042S	S	83	S	82	S	76	S	72	S	97	S	90

R (Resistant reaction) = <10% disease incidence

S (Susceptible reaction) = ≥ 10% disease incidence

pathologists at ICRISAT, Patancheru. The universally downy mildew susceptible genotype 7042S was used for maintenance and increase of isolates in a greenhouse whenever necessary.

3.2 METHODS

3.2.1 General

Common methodologies are described under this head. The finer details wherever needed are given in the respective heads. Every experiment was repeated at least once for confirmation.

3.2.1.1 Sterilization of potting mixture

Potting mixture containing Alfisol, farmyard manure and sand in a proportion of 3:2:2 (v/v/v) was sterilized in an autoclave at 6.80 kg pressure for 2 h/day on two consecutive days.

3.2.1.2 Surface-sterilization of seed

Seeds of all the host genotypes were surface-sterilized with 2 per cent sodium hypochlorite (NaOCl) for five minutes, washed thoroughly with sterilized distilled water and dried at room temperature (approximately 25°C) prior to sowing.

3.2.1.3 Maintenance of isolates

All the isolates used in the study were maintained on pot-grown seedlings of a highly susceptible genotype 7042S through asexual generations. Infected plants of each isolate were kept separately in an individual polyacrylic isolation chambers (Plate 1) measuring 60cm X 60cm X 90cm in a greenhouse at 25 ± 2°C. Each isolate was inoculated onto a fresh set of 7042S seedlings, once a month,

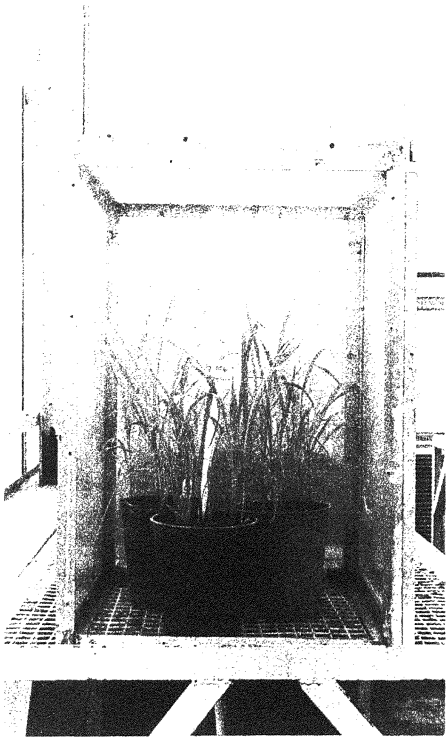

Plate 1 : A polyacrylic isolation chamber with downy mildew infected pearl millet plants of 7042S inside in a greenhouse at 25±2°C at ICRISAT, Patancheru

with sporangia from the previous generation. Plants were kept free from insect pests and other diseases and were adequately fertilized and watered. The sporangial inocula for experiments were collected from these systemically infected plants.

3.2.1.4 Preparation of inoculum

Infected leaves from individual isolates were collected, excised into pieces and washed in running tap water, using a cotton swab to remove old downy growth from the leaf surface. These leaf pieces were wiped dry with tissue paper and placed with their abaxial surfaces up in plastic-tray humidity chambers lined with moist blotting paper. The humidity chambers were incubated in dark at 20°C for 6 h. The incubator was programmed in such a way that the temperature was reduced to 2°C after 6 h of incubation in order to prevent the release of zoospores from mature sporangia until the sporangial collection next day. Sporangia from sporulated leaves were harvested into ice-cold (4°C) sterilized distilled water, separately for each isolate, using a soft camel hair brush. The sporangial suspension was filtered through a double layered muslin cloth to remove conidiophores and other particles. The concentration of sporangia was measured using a haemocytometer and adjusted to a desired concentration.

3.2.1.5 Inoculation technique

Potted seedlings were spray-inoculated with sporangial suspension at the coleoptile to first-leaf stage using a hand sprayer in the inoculation chamber and covered immediately with moist polyethylene sheet to provide >95% relative humidity necessary for infection. Inoculated seedlings were incubated in the dark

at 20°C for 24 h. The pots were then transferred onto greenhouse bench where temperature was maintained at 25 ± 2°C (Plate 2).

3.2.1.6 Preparation of leaf pieces for microscopy

Necrotic leaf pieces collected form infected plants were surface-sterilized with 2 per cent NaOCl and washed thoroughly with sterilized distilled water. These leaf pieces were cleared by incubating at 40°C in 5 per cent NaOH for 12-16 h. Cleared leaf pieces were rinsed in distilled water and observed under microscope using a 10X objective for the presence of oospores.

3.2.2 Detection of pathogenic variability among isolates of *S. graminicola*

3.2.2.1 Establishment of parental isolates form oosporic inocula

Isolates were established from oospores of *S. graminicola* contained in leaf powder samples of pearl millet. Plastic pots of 15cm diameter were filled with autoclaved potting mixture. The potting mixture in each pot was infested by mixing 1 g of oospore-bearing leaf powder in the top 5cm layer of the mix. Surface-sterilized seeds of the universally susceptible pearl millet genotype 7042S were sown @ 25 seeds per pot. Three pots were maintained for each of the 21 inoculum sources. Pots for each inoculum were kept separately in polyacrylic isolation chambers in a greenhouse at 25 ± 2°C to avoid any cross contamination. Pots were watered regularly and observed daily for infection. One month after inoculation, all the infected seedlings were retained and healthy seedlings were uprooted. Sporangia from these infected seedlings were bulked and used for subsequent inoculation of seedlings for isolate maintenance. Isolates thus established from oosporic inocula were denoted as parental isolates.

Plate 2 : Sets of downy mildew inoculated seedlings in pots on greenhouse benches at 25±2°C at ICRISAT, Patancheru

Data recording

Data were recorded for latent period (time in days from inoculation to sporulation) and disease incidence (percentage of infected seedlings). For latent period, data recording began 6th day after inoculation and continued until day 30. Number of infected seedlings and total seedlings per pot were recorded 30 days after inoculation (DAI) to calculate the per cent disease incidence.

"Latent period" was expressed as the number of days, when about 50 per cent of the infected seedlings showed sporulation (Thakur *et al.*, 1998b).

3.2.2.2 Evaluation of parental isolates for pathogenicity on host differentials

Twenty-one parental isolates were evaluated against ten host differentials for variation in pathogenicity. Seeds of the ten host differentials sown in 10cm diameter plastic pots filled with autoclaved potting mixture. For a single isolate, each host genotype was maintained in three replications with 100 seedlings per replication. The seedlings were inoculated with the sporangial suspension (5 x 10^5 sporangia ml^{-1}) of each isolate as described above.

Data recording

Data were recorded for latent period, disease incidence and oospore production per unit area. To determine the latent period, seedlings were observed daily for infection. Data recording for latent period began 5th day after inoculation and continued until day 11. Number of infected seedlings and total seedlings per pot were recorded 15 DAI to calculate the per cent disease incidence. To determine the oospore production, necrotic leaf portions from five infected seedlings of each genotype were collected 30 to 45 DAI.

To determine the quantitative differences in virulence levels of the isolates, virulence index (Thakur and Rao, 1997) was calculated as follows :

$$\text{Virulence index} = \text{Per cent disease incidence} \times \frac{1}{\text{Latent period}}$$

The twenty one parental isolates were classified into seven pathotype groups based on similarities in pathogenicity reaction on the 10 host differentials and a representative isolate from each group (Sg 048, Sg 149, Sg 021, Sg 110, Sg 153, Sg 139 and Sg 152) was identified for further studies.

3.2.2.3 Estimation of oospore production

Necrotic leaf pieces collected from five infected seedlings of each host genotype were dried under shade in brown paper bags, and stored at room temperature (approximately 25°C) until observation. Leaf pieces measuring 1 X 1 cm^2 were cleared as described earlier and examined under microscope, for the presence of oospores. In each replication, for each host genotype 10 leaf pieces were observed. Oospore production rating was scored on a modified 1-4 rating scale where 1 = No oospores, 2 = 1 to 100 oospores/cm^2, 3 = 101-1000 oospores/cm^2 and 4 = > 1000 (numerous) oospores/cm^2 of leaf area (Thakur and Shetty, 1993).

3.2.2.4 Establishment of single-zoospore isolates from parental isolates

Single-zoospore isolates (SZIs) were established form the representative isolates of the seven pathotype groups (Sg 048, Sg 149, Sg 021, Sg 110, Sg 153, Sg 139 and Sg 152). Inoculum from each isolate was prepared as described earlier.

The spore suspension thus obtained was diluted and adjusted to a concentration having 2-3 sporangia per field of microscope. This diluted suspension was kept at 25°C for about 30 minutes to allow the release of zoospores from sporangia. A small amount (0.5 ml) of zoospore suspension was spread uniformly over the surface of sterile water agar medium (1%) in petriplates and the excess suspension was drained off. Single, well-isolated zoospores were marked on water agar using a dummy objective (10X) under the microscope. Single zoospores were picked up with the help of a flat-tipped needle and transferred onto the emerging coleoptile of pearl millet seedlings (7042S) grown in 5cm diameter pots. Pots were then covered with polyethylene bags and incubated overnight at 20°C. Plants were observed daily for symptoms. The infected seedlings were immediately kept in polyacrylic isolation chambers and the uninfected seedlings were discarded. Likewise, a total of 70 SZIs (10 SZIs from each isolate) were established and maintained separately under controlled conditions in a greenhouse.

3.2.2.5 Evaluation of single-zoospore isolates (SZIs) for pathogenicity on host differentials

Instead of testing all the 70 SZIs, 10 SZIs developed from a highly virulent parental isolate Sg 139 and another 10 SZIs developed from a weakly virulent isolate, Sg 110 were selected for evaluation of their pathogenicity reaction on host differentials. The experiment was conducted as described in 3.2.2.2.

Data recording

The data were recorded for latent period and disease incidence as described in 3.2.2.2.

3.2.3 Assessment of genetic diversity among isolates of *S. graminicola*

The genetic diversity among 21 parental isolates of *S. graminicola* was assessed using AFLP markers, a more advanced DNA fingerprinting technique (Vos *et al.*, 1995).

3.2.3.1 DNA extraction

Due to an unidentified, viscous material co-precipitating with DNA, several DNA extraction procedures were tried. Finally, the DNA from 21 parental isolates was extracted in pure form by modifying the procedures described by Hulbert and Michelmore (1988), Cenis (1992) and Sastry *et al.* (1995). The modified protocol was as follows : the sporangia of *S. graminicola* from sporulated leaves were harvested into ice-cold sterile distilled water. The sporangial suspension was centrifuged in 1.5 ml microfuge tubes at 4°C, at 1000 rpm for 5 minutes to get sporangia in pellet form. About 150 mg of sporangial pellet was used to isolate genomic DNA. The pellet was washed twice with phosphate saline buffer and centrifuged at 500 rpm for 10 minutes. The supernatant was decanted and 600 µl of extraction buffer (0.5 M Tris-HCl, pH 8.0, 0.5 M EDTA, pH 8.0 and 20% SDS) and 20 µl of proteinase K were added to the pellet. The contents were mixed gently and incubated at 65°C for 20 minutes. After cooling the contents to room temperature, these were centrifuged at 10000 rpm, for 10 minutes. Supernatant was transferred to a fresh tube, mixed with 15 µl of RNase A (10 mg/ml) and incubated at 37°C for 1 hour. An equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added, mixed gently and centrifuged at 10000 rpm for 5 minutes. The aqueous layer was removed and the above step was repeated once. The aqueous phase was once again removed and extracted with an equal volume of

chloroform - isoamyl alcohol (24:1) as above. DNA was precipitated by adding 0.1 volume of 0.3 M sodium acetate and an equal volume of chilled isopropanol. The DNA pellet was washed twice with 70% ethanol, dried at room temperature and dissolved in $T_{10}E_1$ (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0).

3.2.3.2 AFLP analysis

AFLP analysis was performed as described by Vos *et al.* (1995). Research kit for AFLP of genomic DNA was from Life Technologies, USA and assays were carried out as described in the manufacturer's protocol. Although a number of primer combinations were tested, the results reported in the present study were obtained with three primer combinations. The two *Eco*RI (E-TG and E-TT) primers and three *Mse*I (M-CAT, M-TAG and M-CTA) primers were used in these three combinations (E-TG/M-CAT, E-TT/M-TAG and E-TG/M-CTA) for amplication. Genomic DNA (200 ng) was incubated with 1U *Eco*RI/*Mse*I mix for 90 minutes at 37°C with 1X Buffer. Digestion was followed by inactivation at 70°C for 15 minutes, the aliquot was distributed in two equal parts for ligation. Solution containing equimolar concentration of Adaptors as given in the kit and T4 DNA ligase was mixed and incubated at 20°C for 2 hours. The ligated sample was diluted to 10-fold and 2 µl was used for pre amplification with 8 µl pre amplification mixture, 1U Taq polymerase (Promega Corporation Wis.) and 1X buffer. Selective amplification was carried out with 50-fold diluted preamplified mix, using [γ^{32}P]-ATP labeled selectively modified *Eco*RI primer, selective *Mse*I primer containing dNTP mix, 1U Taq polymerase, 1 X Buffer and AFLP grade water as described in the protocol. Reaction cycles were carried out on Perkin Elmer 9600 Thermocycler. The amplified samples were mixed with 98 per cent

formamide and xylene cyanol-bromophenol blue dyes, heated at 96°C for 5 minutes, ice-cooled and loaded on 6 per cent polyacrylamide gel at 80 W according to standard method described for DNA sequencing (Sambrook *et al.*, 1989). After electrophoresis, autoradiograms were obtained using Kodak X-Omat films. The dried gels were placed with the X-ray films in cassettes overnight at room temperature. Amplification products were viewed on by autoradiographs and scored for polymorphism. Experiment with each primer combination was repeated a minimum of 2 times to establish the consistency of the bands.

3.2.4 Identification of mating types among isolates of *S. graminicola*

3.2.4.1 Assay for mating types

The 70 SZIs derived from the seven representative isolates of pathotype groups were tested for their oospore formation potential when inoculated alone on seedlings of cultivar 7042S under greenhouse conditions. Based on oospore formation these SZIs were classified broadly into two groups viz., self-sterile and self-fertile isolates. Of the self-sterile isolates identified, 10 SZIs (Sg 139-1 through Sg 139-10), derived from the highly virulent parental isolate Sg 139, and six SZIs (Sg 110-1, Sg 110-3, Sg 110-4, Sg 110-5, Sg 110-8 and Sg 110-10) derived from the weakly virulent parent isolate Sg 110, were tested for cross compatibility by inoculating the isolates alone (16 isolates) and in all possible combinations (10 X 6) on 7042S to determine the mating types. Sporangial inocula (5 X 10^5 sporangia ml^{-1}) from both the isolates were mixed in equal (1:1) proportions before inoculation and pairwise inoculations were done among the 16 selected SZIs. For each combination and single isolate inoculations, two pots with 50 seedlings per pot were maintained, and 10 leaf pieces of 1 X 1 cm^2 in size were

collected randomly from seedlings in each pot and examined microscopically for the presence of oospores. All the isolates and isolate combinations were maintained separately in isolation chambers to avoid any cross contamination.

3.2.4.2 Composition, frequency and distribution of mating types

The 70 SZIs, irrespective of their fertility, were cross inoculated with PT2 (mating type Mat A) and PT3 (mating type Mat B), the two known standard mating type isolates of *S. graminicola* (Rao *et al.*, 1994). Sporangial inocula (5 X 10^5 sporangia ml^{-1}) from two isolates of a combination were mixed in 1:1 proportion and inoculated on seedlings of cultivar, 7042S. For each combination two pots with 50 seedlings per pot were maintained and kept separately in isolation chambers under greenhouse conditions. Leaf samples were collected as above and examined microscopically for the presence of oospores.

3.2.5 Inheritance of virulence in *S. graminicola*

3.2.5.1 Selection of parents and tester host genotype

The highly virulent SZI, Sg 139-4 (mating type Mat A) and weakly virulent SZI (but avirulent on IP 18292), Sg 110-9 (mating type Mat B) were selected as parents for the study of inheritance of virulence in *S. graminicola*. As the two selected parents exhibited quite distinct virulence phenotypes on the pearl millet genotype, IP 18292 hence, it was selected as a tester host genotype for the study.

3.2.5.2 Hybridization

A cross was made between Sg 139-4 and Sg 110-9 on pearl millet cultivar 7042S which is highly susceptible to both the isolates. The seedlings of 7042S

were spray-inoculated with a sporangial suspension (5 X 10^5 sporangia ml^{-1}) containing inocula of the parents in equal (1:1) proportion. Infected seedlings were allowed to produce oospores under greenhouse conditions and 50 pots of 15cm diameter with 10 seedlings per pot were maintained to get sufficient F_1 oospores.

3.2.5.3 Establishment of F_1 progeny

One month after inoculation, necrotic leaves from all the infected plants were collected, dried and ground into powder. The leaf powder thus obtained contained F_1-oospores. These F_1-oospores were raised into F_1-sporangia through soil inoculation. A low frequency of infection from F_1-oospores was obtained by adding 0.25 g of leaf powder containing oospores to the autocalved soil contained in 15cm diameter pots that were sown with 7042S. As infected seedlings occurred infrequently and rarely, each infected seedling was assumed to have infection from an individual oospore. Sporangia from each seedling were maintained separately on 7042S as an individual F_1-progeny isolate in isolation chambers in a greenhouse.

3.2.5.4 Evaluation of F_1 progeny

A total of 33 F_1-progeny isolates were established and each progeny isolate was tested for virulence phenotype (pathogenicity reaction) in terms of disease incidence on 100 seedlings of the tester host genotype, IP 18292. Each time a group of F_1 progeny isolates (usually 10 isolates) along with their original parents, Sg 139-4 and Sg 110-9 were tested. However, no segregation was found in F_1 generation and all the F_1-progeny isolates exhibited only avirulent reaction.

3.2.5.5 Establishment of F_2 progeny

F_1-progeny isolates were allowed to produce oospores on 7042S. As all the F_1 progeny isolates exhibited similar type of avirulence on tester host genotype IP 18292, the necrotic leaves of 7042S collected from all F_1 progeny isolates were bulked, dried and ground into leaf powder which contained F_2-oospores. From these F_2-oospores, F_2-sporangia were obtained as above on 7042S. A total of 230 single-oospore F_2-progeny isolates were established and maintained separately on seedlings of 7042S in isolation chambers.

3.2.5.6 Evaluation of F_2 progeny (segregating generation)

Each F_2 progeny isolate was tested for its virulence phenotype in terms of disease incidence on 100 seedlings of the tester host genotype IP 18292. Based on disease incidence (DI), all F_2 progeny isolates were classified into two virulence phenotype groups viz., virulence reaction type (>10% DI) and avirulence reaction type (<10% DI), and the data obtained was used to assess the segregation pattern. Each time the virulence phenotypes of a group of F_2 progeny isolates (usually ten isolates) were tested. The two original parents, Sg 139-4 and Sg 110-9, were also tested to serve as checks.

3.2.5.7 Backcrosses

Of the 33 F_1 progeny isolates, two isolates with different compatible mating types were identified and crossed with the original parents of complementary mating type. Forty-six progeny isolates from backcross 1 (F_1 X Sg 110-9) and 62 progeny

isolates from backcross 2 (F_1 X Sg 139-4) were recovered and evaluated for virulence phenotype on the tester host genotype, IP 18292.

A schematic representation of various steps involved in the study of inheritance of virulence in *S. graminicola* is given in Figure 2.

3.3 STATISTICAL ANALYSIS

3.3.1 Pathogenic variability

The experiments to detect pathogenic variability among the isolates of *S. graminicola* were conducted in factorial completely randomized design under greenhouse conditions. The data on latent period, disease incidence, and virulence index were subjected to Analysis of Variance (Gomez and Gomez, 1984) using GENSTAT statistical package (Rothamsted Experiment Station, Herpenden, Herts AL 52 JQ, UK), to determine significant differences among isolates, host genotypes and their interactions. Average Linkage Cluster analysis was done using the Euclidian test to determine the similarity among the isolates and to classify them to pathotype groups based on per cent disease incidence. A correlation analysis was done to determine the relationships among the latent period and disease incidence (Gomez and Gomez, 1984).

3.3.2 Genetic variability

The relatedness of the 21 parental isolates of *S. graminicola* was estimated by means of scoreable bands form three primer combinations used in AFLP analysis. Differences in banding pattern were scored on the basis of presence or absence of a band. Similarities between the DNA fingerprints were calculated using Nei and Lei's (1979) similarity index, given by the formula $S_{(xy)} = 2N_{xy} / (N_x + N_y)$, where

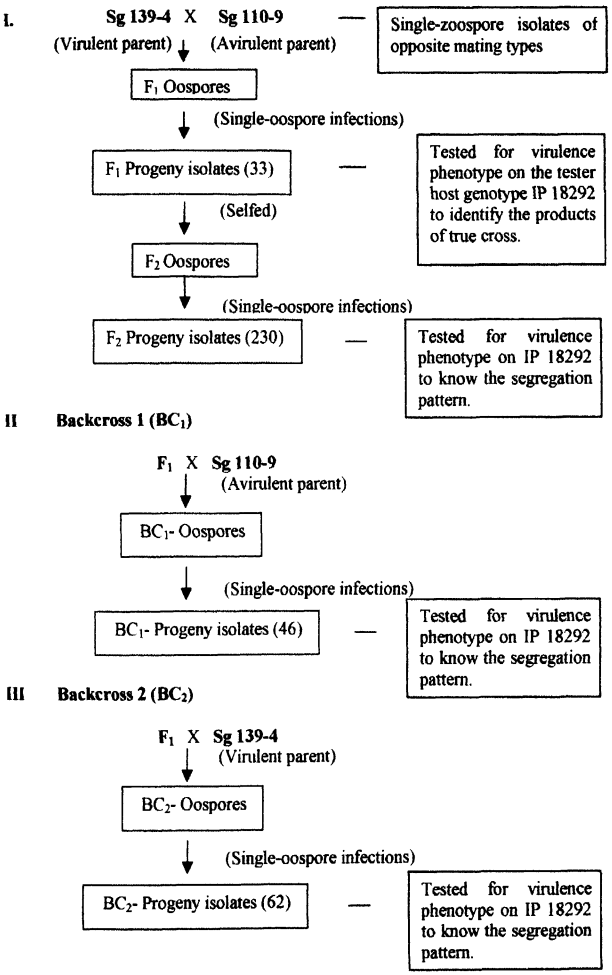

Fig. 2 : Schematic representation of inheritance of virulence study in *Sclerospora graminicola*

N_{xy} is the number of shared fragments and N_x and N_y are the number of fragments in the fingerprints x and y, respectively. Based on similarity index values, a cluster analysis was performed using the statistical software package SYSTAT 5.1. A dendrogram showing the mean similarities between groups of different isolates was generated.

3.3.3 Compatible mating types

The goodness-of-fit of ratio of mating types, Mat A and Mat B, was subjected to Chi-square analysis (Panse and Sukhatme, 1978).

3.3.4 Inheritance of virulence

The goodness-of-fit of the segregation ratio of virulence phenotypes to theoretical ratio was tested using Chi-square analysis (Panse and Sukhatme, 1978).

Results

CHAPTER - IV

RESULTS

Results of the experiments conducted in the present investigation on pathogenic variability, genetic diversity, identification of mating types and inheritance of virulence in *S. graminicola*, the incitant of downy mildew in pearl millet are presented here under.

4.1 CHARACTERIZATION OF ISOLATES OF *S. graminicola* FOR PATHOGENIC VARIABILITY

4.1.1 Variation in pathogenicity of parental isolates

Data were recorded for disease incidence and latent period while generating asexual inocula from oosporic inocula of the 21 parental isolates, on the susceptible host genotype 7042S (Table 3). Highly significant differences were observed among the isolates for both, disease incidence and latent period. Of the 21 parental isolates, the highest disease incidence was recorded in isolate Sg 153 (76.15 %) followed by Sg 139 (69.25 %) and Sg 152 (65.04 %), and the least was recorded in Sg 021 (1.97 %), followed by Sg 004 (2.61 %) and Sg 026 (8.41 %). No significant difference was observed between Sg 021 and Sg 004 for disease incidence. Isolates collected during 1997 recorded significantly high disease incidence than those collected in the previous years, and an increasing trend was observed from the year 1988 to 1997. Isolates with higher disease incidence recorded shorter latent period and vice versa. A high latent period of 30 days was observed in isolates Sg 004 and Sg 021, followed by 28 days in Sg 026 and 25 days in Sg 015 while, the least was

Table 3 : **Pathogenicity of 21 parental isolates of *Sclerospora graminicola* on susceptible host genotype 7042S, while generating asexual inocula from oosproric inocula**

Isolates	Disease incidence (%)[a]	Latent period (day)[a]
Sg 004	2.61 (9.23)[b]	30.00
Sg 015	12.57 (20.76)	25.00
Sg 021	1.97 (8.01)	30.00
Sg 025	20.61 (27.00)	23.33
Sg 026	8.41 (16.84)	28.00
Sg 032	25.16 (30.10)	21.00
Sg 040	34.87 (36.19)	19.67
Sg 045	23.7 (29.13)	23.00
Sg 046	24.33 (29.56)	22.67
Sg 047	13.01 (21.13)	24.00
Sg 048	36.16 (39.97)	14.33
Sg 088	50.45 (45.26)	10.33
Sg 110	41.67 (40.20)	19.00
Sg 115	46.53 (43.01)	15.00
Sg 139	69.25 (56.32)	6.67
Sg 140	55.36 (48.08)	12.00
Sg 149	52.61 (46.50)	14.67
Sg 150	59.57 (50.52)	11.00
Sg 151	64.68 (53.54)	9.33
Sg 152	65.04 (53.76)	8.00
Sg 153	76.15 (60.77)	6.00
S.Ed. ±	0.5648	1.6777
CD 1%	1.5240	4.5272

[a] : Mean of three replications

[b]: Figures in parentheses are arcsin transformed values

observed in isolate Sg 153 (6.00 days), followed by Sg 139 (6.67 days) and Sg 152 (8.00 days). However, there was no significant difference between isolates Sg 004 and Sg 026, and isolates Sg 153, Sg 139 and Sg 152 for latent period (Table 3).

4.1.2 Evaluation of parental isolates for pathogenicity on host differentials

The data on per cent disease incidence, disease reaction, latent period, virulence index and oospore production of the 21 parental isolates of *S. graminicola* on 10 differential host genotypes are presented in Tables 4-8.

4.1.2.1 Variation in virulence

All 21 isolates induced symptoms on host genotypes 7042S, P 536-2, P 1564, P 2895-3 and 700481-21-8 and therefore were considered virulent on these host genotypes. However, majority of isolates failed to cause disease on IP 18297 and IP 18292 and so these were considered avirulent on these two genotypes, while few isolates exhibited similar avirulent reaction on the remaining host genotypes (Table 4).

Irrespective of the isolate used, symptoms induced on IP 18292 and P 1564 are severe stunting of the seedling, dark green foliage and lack of sporulation, while those on other host differentials include chlorosis coupled with ample sporulation. This suggests that the symptom expression on any host genotype by any isolate is host specific but not isolate specific.

Table 4 : Per cent disease incidence[a] of 21 parental isolates of *Sclerospora graminicola* on ten differential host genotype

Isolates	Differential host genotypes										Mean
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 004	20 41	3 68	0 00	40 03	18 03	19 91	0 75	33 42	44 26	96 84	27 73
	(26 84)[b]	(11 02)	(0 00)	(39 25)	(25 11)	(26 49)	(4 97)	(35 32)	(41 70)	(79 76)	(29 05)
Sg 015	1 49	1 22	0 85	46 56	55 07	0 41	0 63	46 21	0 28	95 43	24 82
	(6 99)	(6 34)	(5 23)	(43 03)	(47 91)	(3 67)	(4 54)	(42 83)	(2 91)	(77 70)	(24 11)
Sg 021	0 00	24 00	0 27	31 15	15 59	2 29	0 00	26 58	2 18	94 41	19 65
	(0 00)	(29 32)	(2 88)	(33 92)	(23 25)	(8 69)	(0 00)	(31 03)	(8 49)	(76 37)	(21 40)
Sg 025	11 79	0 00	0 00	28 28	12 36	33 48	0 00	34 90	1 13	85 74	20 77
	(20 00)	(0 00)	(0 00)	(32 12)	(20 52)	(35 35)	(0 00)	(36 21)	(6 10)	(65 87)	(21 84)
Sg 026	0 00	2 25	0 00	38 42	0 76	0 83	0 19	25 09	0 21	92 58	16 03
	(0 00)	(8 59)	(0 00)	(38 30)	(5 00)	(5 14)	(2 49)	(30 05)	(2 11)	(74 20)	(16 58)
Sg 032	15 34	7 06	0 00	43 94	10 25	29 50	0 85	53 72	0 00	97 62	25 83
	(23 05)	(15 40)	(0 00)	(41 52)	(18 65)	(32 90)	(5 20)	(47 14)	(0 00)	(81 19)	(26 50)
Sg 040	9 92	0 49	0 27	25 58	55 29	27 93	0 20	48 69	0 76	94 00	26 31
	(18 33)	(3 86)	(2 40)	(30 37)	(48 04)	(31 90)	(2 06)	(44 25)	(4 96)	(75 85)	(26 20)
Sg 045	60 86	7 97	0 00	34 52	75 24	49 75	6 62	24 28	0 43	94 03	35 37
	(51 28)	(16 37)	(0 00)	(35 98)	(60 17)	(44 86)	(14 89)	(29 52)	(3 03)	(75 93)	(33 20)
Sg 046	0 17	1 76	0 57	47 45	2 61	0 21	0 98	48 72	0 00	96 47	19 89
	(2 36)	(7 56)	(4 07)	(43 54)	(9 30)	(2 58)	(5 68)	(44 27)	(0 00)	(79 21)	(19 86)
Sg 047	34 71	13 95	0 00	50 50	46 84	46 31	3 31	42 89	23 54	96 73	35 88
	(36 09)	(21 89)	(0 00)	(45 29)	(43 19)	(42 88)	(10 42)	(40 91)	(28 10)	(79 64)	(34 93)
Sg 048	45 11	13 98	0 00	50 25	28 04	49 63	0 85	32 74	61 27	97 61	37 95
	(42 19)	(21 94)	(0 00)	(45 14)	(31 97)	(44 79)	(5 14)	(34 90)	(51 52)	(81 25)	(35 88)
Sg 088	71 89	22 59	0 00	40 86	74 33	61 34	4 07	88 99	0 00	94 31	45 84
	(58 00)	(28 37)	(0 00)	(39 73)	(59 59)	(51 55)	(11 59)	(70 67)	(0 00)	(76 38)	(39 59)
Sg 110	0 44	0 00	0 44	33 13	0 38	0 38	0 00	17 64	0 00	90 60	14 30
	(3 79)	(0 00)	(3 80)	(35 14)	(3 43)	(3 50)	(0 00)	(24 83)	(0 00)	(72 21)	(14 67)
Sg 115	2 77	34 51	0 00	30 78	33 35	5 05	0 00	45 53	1 15	90 97	24 41
	(9 45)	(35 98)	(0 00)	(33 70)	(35 27)	(12 94)	(0 00)	(42 44)	(5 98)	(72 53)	(24 83)
Sg 139	45 81	13 93	0 00	46 39	86 85	51 55	7 92	59 38	89 55	93 45	49 48
	(42 60)	(21 92)	(0 00)	(42 93)	(68 79)	(45 89)	(16 34)	(50 41)	(71 15)	(75 20)	(43 52)
Sg 140	27 69	9 07	0 36	32 13	23 65	36 17	3 79	73 77	0 00	96 43	30 31
	(31 73)	(17 53)	(3 43)	(34 53)	(29 10)	(36 95)	(11 22)	(59 19)	(0 00)	(79 19)	(30 29)
Sg 149	0 74	1 01	0 00	54 18	8 10	2 14	0 26	26 73	1 96	95 00	19 01
	(4 87)	(5 77)	(0 00)	(47 40)	(16 53)	(8 41)	(2 35)	(31 13)	(8 01)	(77 12)	(20 16)
Sg 150	67 30	12 63	0 00	40 60	75 82	45 09	3 64	79 19	0 00	97 88	42 22
	(55 12)	(20 78)	(0 00)	(39 58)	(60 55)	(42 18)	(10 93)	(62 87)	(0 00)	(81 63)	(37 36)
Sg 151	19 83	4 54	0 00	45 93	38 70	46 57	5 65	57 35	61 54	97 25	37 74
	(26 44)	(12 29)	(0 00)	(42 67)	(38 47)	(43 03)	(13 75)	(49 23)	(51 68)	(80 59)	(35 81)
Sg 152	14 04	0 00	0 00	4 05	94 49	53 58	0 00	56 40	0 00	87 02	30 96
	(22 00)	(0 00)	(0 00)	(11 55)	(76 46)	(47 05)	(0 00)	(48 68)	(0 00)	(68 88)	(27 46)
Sg 153	51 92	17 82	0 00	45 12	86 72	49 16	18 72	55 44	0 00	97 01	44 19
	(46 10)	(24 95)	(0 00)	(42 20)	(68 63)	(36 27)	(25 63)	(48 12)	(0 00)	(80 14)	(39 20)
Mean	23 92	9 16	0 13	38 56	40 12	30 06	2 78	46 56	13 73	94 35	
	(25 11)	(14 76)	(1 04)	(37 99)	(37 62)	(29 859)	(7 01)	(43 05)	(13 65)	(76 80)	

	S.Ed. ±	CD 1%
Isolates	0 2800	0 7214
Host genotypes	0 1932	0 4978
Interaction	0 8855	2 2811

[a] Mean of three replications

[b] Figures in parentheses are arcsin transformed values

4.1.2.2 Variation in aggressiveness

Considerable variation was found among the isolates of *S. graminicola* across the host differentials for disease incidence, ranging from 0.17 with the isolate Sg 046 on IP 5272-1 to 97.88 with the isolate Sg 150 on 7042S (Table 4). Among the 21 isolates evaluated, highest mean disease incidence was recorded with the isolate Sg 139 (49.48 %), followed by Sg 088 (45.84 %) and the lowest was observed with the isolate Sg 110 (14.30 %), across the host-genotypes. Most of the isolates were found highly aggressive on 7042S, moderately aggressive on 700481-21-8, P 1564 and P 536-2, and least aggressive on IP 18297. However, on other host genotypes, the disease incidence was highly variable within and across the isolate-genotype combinations (Table 4). The analysis of variance indicated highly significant ($P<0.01$) effects of isolates, host genotypes and their interactions on disease incidence (Appendix I).

4.1.2.3 Variation in disease reaction

All isolates showed susceptible reaction on host genotypes 7042S and 700481-21-8 and resistant reaction on IP 18297 (Table 5). Except isolate Sg 152 on host genotype P 536-2 and isolate Sg 153 on P 3281-1, all other isolates gave differential reactions on these host genotypes. However, isolates had clear differential disease reactions on IP 5272-1, IP 18296, P 1564, P 2895-3 and IP 18292 (Table 5).

Table 5 : Disease reaction of 21 parental isolates of *Sclerospora graminicola* on ten differential host genotypes

Isolates	Differential host genotypes									
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S
Sg 004	S	R	R	S	S	S	R	S	S	S
Sg 015	R	R	R	S	S	R	R	S	R	S
Sg 021	R	S	R	S	S	R	R	S	R	S
Sg 025	S	R	R	S	S	S	R	S	R	S
Sg 026	R	R	R	S	R	R	R	S	R	S
Sg 032	S	R	R	S	S	S	R	S	R	S
Sg 040	R	R	R	S	S	S	R	S	R	S
Sg 045	S	R	R	S	S	S	R	S	R	S
Sg 046	R	R	R	S	R	R	R	S	R	S
Sg 047	S	S	R	S	S	S	R	S	S	S
Sg 048	S	S	R	S	S	S	R	S	S	S
Sg 088	S	S	R	S	S	S	R	S	R	S
Sg 110	R	R	R	S	R	R	R	S	R	S
Sg 115	R	S	R	S	S	R	R	S	R	S
Sg 139	S	S	R	S	S	S	R	S	S	S
Sg 140	S	R	R	S	S	S	R	S	R	S
Sg 149	R	R	R	S	R	R	R	S	R	S
Sg 150	S	S	R	S	S	S	R	S	R	S
Sg 151	S	R	R	S	S	S	R	S	S	S
Sg 152	S	R	R	R	S	S	R	S	R	S
Sg 153	S	S	R	S	S	S	S	S	S	S

R : Resistant reaction (<10% disease incidence)
S : Susceptible reaction (≥10% disease incidence)

4.1.2.4 Variation in latent period

The isolates varied significantly for latent period, ranging from 5 to 11 days in various isolate-genotype combinations (Table 6). Across the isolates, the mean latent period was longest on IP 18292 (9.39 days) and shortest on 7042S (5.90 days), while across the host genotypes it was longest for isolate Sg 110 (9.49 days) and shortest for isolate Sg 139 (7.08 days). There were significant ($P<0.01$) effects of host genotypes, isolates and their interactions on latent period (Appendix II). A significant negative correlation ($r = -0.77$ at $P<0.01$) was found between latent period and disease incidence across the host genotypes and isolates.

4.1.2.5 Variation in virulence index

Virulence index of the 21 parental isolates ranged between 0.02 and 18.69 (Table 7). Virulence index was generally greater for most isolates on 7042S, moderate on 700481-21-8, P 536-2 and P 1564, and lower on IP 18297. Across host genotypes, isolate Sg 139 had greatest virulence index (7.55) and Sg 110 the lowest (2.04), while across isolates it was highest on 7042S (16.09) and lowest on IP 18297 (0.02). F values were highly significant ($P<0.01$) for isolates, host genotypes and their interaction (Appendix III).

4.1.2.6 Variation in oospore production

There was no oospore production in host genotypes P 1564 and IP 18292 with any of the 21 isolates, and it was highest (3.79 on a 1-4 scale) for most of the isolates on 7042S (Table 8). However, considerable variation was

Table 6: Latent period[a] of 21 parental isolates of *Sclerospora graminicola* on ten differential host genotypes

Isolates	Differential host genotypes										Mean
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 004	9.33	9.00	-	8.00	10.44	9.50	7.76	9.50	8.50	5.56	8.62
Sg 015	10.20	10.33	8.00	6.67	7.83	10.00	10.00	7.00	10.50	5.67	8.62
Sg 021	-[b]	8.45	10.00	9.33	10.16	10.80	-	8.00	9.67	5.67	9.01
Sg 025	8.33	-	-	8.16	9.67	8.50	-	9.00	11.00	6.66	8.76
Sg 026	-	10.50	-	8.00	10.33	9.66	9.00	8.83	10.25	6.33	9.11
Sg 032	8.77	9.16	-	9.00	8.44	8.50	10.00	7.72	-	6.00	8.45
Sg 040	6.50	9.50	8.17	7.39	10.56	6.61	11.00	8.33	10.00	6.50	8.46
Sg 045	7.33	8.50	-	8.05	7.83	8.55	7.56	9.50	10.08	6.16	8.17
Sg 046	11.00	9.50	7.56	7.67	9.50	10.00	9.67	8.22	-	5.83	8.77
Sg 047	7.83	9.00	-	7.00	8.83	8.50	9.50	8.33	9.16	6.00	8.24
Sg 048	8.00	8.00	-	7.16	8.78	7.39	8.00	8.50	7.50	6.00	7.70
Sg 088	6.44	7.24	-	7.00	8.00	7.61	8.27	7.00	-	5.28	7.11
Sg 110	10.00	-	9.08	9.61	10.50	10.50	-	10.67	-	6.00	9.49
Sg 115	9.16	9.67	-	7.56	10.00	7.05	-	8.33	9.50	6.50	8.48
Sg 139	7.50	8.00	-	7.00	6.56	7.16	8.17	7.33	7.00	5.00	7.08
Sg 140	8.78	8.16	7.13	7.39	9.50	8.56	9.00	7.27	-	5.66	7.93
Sg 149	10.00	9.00	-	7.61	9.50	8.39	9.10	9.50	10.75	6.00	8.88
Sg 150	7.61	9.00	-	7.05	7.50	8.00	9.00	8.05	-	5.89	7.76
Sg 151	7.83	9.16	-	7.89	8.50	8.05	7.61	7.66	8.00	5.66	7.82
Sg 152	8.11	-	-	8.22	6.78	7.44	-	7.72	-	6.00	7.38
Sg 153	7.66	8.16	-	6.44	7.89	7.55	8.00	8.61	-	5.56	7.49
Mean	8.44	8.90	8.33	7.72	8.91	8.49	8.85	8.34	9.39	5.90	

	S.Ed. ±	CD 1%
Isolates	0.0561	0.1446
Host genotypes	0.0387	0.0998
Interaction	0.1775	0.4572

[a]: Mean of three replications

[b]: No symptoms observed

Table 7 : Virulence index[a] (per cent disease incidence X latent period^{-1}) of 21 parental isolates of *Sclerospora graminicola* on ten differential host genotypes

Isolates	Differential host genotypes										Mean
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 004	2 19	0 41	0 00	5 00	1 73	2 10	0 10	3 52	5 21	17 40	3 77
Sg 015	0 15	0 12	0 11	7 00	7 03	0 04	0 06	6 64	0 03	16 86	3 80
Sg 021	0 00	2 83	0 03	3 34	1 54	0 21	0 00	3 34	0 22	16 66	2 82
Sg 025	1 42	0 00	0 00	3 47	1 27	3 95	0 00	3 88	0 10	12 88	2 70
Sg 026	0 00	0 21	0 00	4 80	0 07	0 08	0 02	2 85	0 02	14 65	2 27
Sg 032	1 75	0 77	0 00	4 88	1 21	3 48	0 09	6 96	0 00	16 27	3 54
Sg 040	1 53	0 05	0 03	3 46	5 24	4 22	0 02	5 85	0 07	14 52	3 50
Sg 045	8 30	0 94	0 00	4 29	9 61	5 81	0 87	2 56	0 04	15 28	4 77
Sg 046	0 02	0 19	0 07	6 19	0 28	0 02	0 10	5 93	0 00	16 55	2 94
Sg 047	4 43	1 56	0 00	7 21	5 30	5 45	0 35	5 15	2 57	16 12	4 82
Sg 048	5 65	1 75	0 00	7 02	3 20	6 72	0 11	3 85	8 17	16 27	5 27
Sg 088	11 16	3 12	0 00	5 84	9 32	8 06	0 50	12 71	0 00	17 91	6 88
Sg 110	0 04	0 00	0 05	3 45	0 04	0 04	0 00	1 65	0 00	15 10	2 04
Sg 115	0 30	3 57	0 00	4 07	3 34	0 71	0 00	5 47	0 12	14 06	3 17
Sg 139	6 12	1 74	0 00	6 63	13 25	7 20	0 97	8 11	12 79	18 69	7 55
Sg 140	3 15	1 11	0 05	4 35	2 49	4 23	0 42	10 14	0 00	17 04	4 30
Sg 149	0 08	0 11	0 00	7 12	0 86	0 26	0 03	2 81	0 18	15 91	2 74
Sg 150	8 84	1 40	0 00	5 76	10 11	5 65	0 40	9 83	0 00	16 63	5 86
Sg 151	2 54	0 50	0 00	5 83	4 55	5 78	0 74	7 48	7 72	17 18	5 23
Sg 152	1 73	0 00	0 00	0 50	13 95	7 20	0 00	7 30	0 00	14 50	4 52
Sg 153	6 78	2 18	0 00	7 01	11 00	9 16	2 34	6 44	0 00	17 46	6 24
Mean	3 15	1 08	0 02	5 11	5 02	3 83	0 34	5 84	1 77	16 09	

	S.Ed. ±	CD 1%
Isolates	0 0751	0 1934
Host genotypes	0 1016	0 1335
Interaction	0 4654	0 6117

[a] Mean of three replications

Table 8: Oospore production ratings[a] (based on 1-4[b] rating scale) of 21 parental isolates of *Sclerospora graminicola* on ten differential host genotypes

Isolates	Differential host genotypes									
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 004	3 90	2 97	-	3 40	1 00	3 07	3 17	3 97	1 00	4 00	2 94
Sg 015	2 90	1 87	2 80	2 00	1 00	1 97	1 87	3 17	1 00	3 07	2 16
Sg 021	-[c]	2 73	3 90	3 17	1 00	3 47	-	3 90	1 00	4 00	2 90
Sg 025	3 93	-	-	3 50	1 00	3 10	-	4 00	1 00	4 00	2 93
Sg 026	-	1 87	-	3 13	1 00	3 50	2 00	3 87	1 00	4 00	2 55
Sg 032	2 87	1 80	-	1 87	1 00	2 00	1 93	2 80	-	3 87	2 27
Sg 040	3 90	1 63	4 00	3 53	1 00	3 10	2 57	3 93	1 00	4 00	2 87
Sg 045	4 00	1 97	-	2 93	1 00	3 27	2 00	3 90	1 00	3 97	2 67
Sg 046	3 60	2 00	3 83	3 07	1 00	3 00	2 77	4 00	-	1 00	3 03
Sg 047	4 00	3 33	-	3 47	1 00	3 63	3 00	4 00	1 00	4 00	3 05
Sg 048	3 87	3 43	-	3 67	1 00	3 73	3 13	4 00	1 00	4 00	3 09
Sg 088	4 00	2 70	-	3 37	1 00	3 77	2 13	3 93	-	4 00	3 11
Sg 110	2 10	-	2 80	2 47	1 00	1 90	-	3 37	-	3 33	2 42
Sg 115	2 00	1 73	-	2 17	1 00	1 63	-	3 13	1 00	3 47	2 02
Sg 139	4 00	3 53	-	3 63	1 00	3 10	3 23	4 00	1 00	4 00	3 06
Sg 140	2 73	1 60	2 13	2 47	1 00	1 93	1 63	3 07	-	3 57	2 24
Sg 149	3 00	2 00	-	2 43	1 00	1 77	2 20	2 80	1 00	3 33	2 17
Sg 150	3 97	3 17	-	3 07	1 00	3 30	3 10	4 00	-	4 00	3 20
Sg 151	4 00	3 13	-	3 50	1 00	2 83	3 03	3 93	1 00	4 00	2 94
Sg 152	2 77	-	-	1 83	1 00	1 47	-	2 87	-	2 93	2 15
Sg 153	4 00	3 43	-	3 50	1 00	3 10	3 03	4 00	-	3 97	3 25
Mean	3 45	2 36	3 24	2 96	1 00	2 79	2 40	3 65	1 00	3 79	

	S.Ed. ±	CD 1%
Isolates	0 0187	0 0482
Host genotypes	0 0253	0 0333
Interaction	0 1160	0 1525

[a] Mean of three replications

[b] 1 = No oospore/cm^2
2 = 1-100 oospores/cm^2
3 = 101-1000 oospores/cm^2
4 = >1000 (innumerous) oospores/cm^2

[c] No symptoms observed

observed for oospore production ratings among the isolates on the remaining host genotypes, and it ranged between 1.47 and 4.00. Across the isolates, the highest mean oospore production rating was recorded on 7042S (3.79) followed by 700481-21-8 (3.65) and IP 5272-1 (3.45), and the lowest was on P 1564 and IP 18292 (1.00). Across the host genotypes, it was highest for Sg 153 (3.25), followed by Sg 150 (3.20) and the lowest in Sg 115 (2.02). Oospore production was highly influenced by host lines, isolates and their interactions (Appendix IV).

4.1.2.7 Pathotype grouping

A dendrogram developed from the average linkage cluster analysis based on disease incidence classified the 21 parental isolates at the 90 per cent similarity level into seven major pathotype groups (Fig. 3). Isolates Sg 004, Sg 032, Sg 047, Sg 048, Sg 151, Sg 040 and Sg 140 were in group I; Sg 015 and Sg 149 in group II; Sg 021 and Sg 115 in group III; Sg 025, Sg 026, Sg 046 and Sg 110 in group IV; Sg 045, Sg 153, Sg 088 and Sg 150 in group V. Two isolates, Sg 139 and Sg 152 were not clustered at the 90 per cent similarity level and thus formed separate groups, group VI and group VII, respectively.

4.1.3 Evaluation of single-zoospore isolates (SZIs) of Sg 139, a highly virulent parental isolate for pathogenicity on host differentials

The data on per cent disease incidence, disease reaction, latent period and virulence index of SZI derived from a highly virulent parental isolate Sg 139 are presented in Tables 9-12.

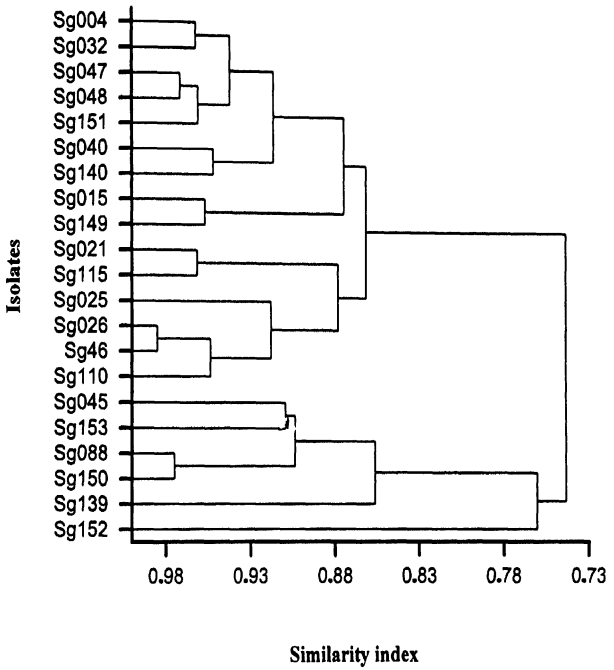

Fig.3: Dendrogram of 21 parental isolates of *Sclerospora graminicola*, based on cluster analysis of disease incidence recorded on 10 differential host genotypes

4.1.3.1 Variation in virulence

All 10 SZI of Sg 139 were found virulent on host genotypes IP 5272-1, P 536-2, P 1564, P 2895-3, 700481-21-8, IP 18292 and 7042S, although they induced different levels of disease incidence (Table 9). However, majority of the isolates did not produce symptoms on IP 18297 and thus were avirulent on this genotype. Isolates Sg 139-1 and Sg 139-10 on IP 18296 and P 3281-1 were found avirulent, while the remaining isolates showed virulent reaction on these genotypes.

4.1.3.2 Variation in aggressiveness

Variation in aggressiveness was clearly evident among the isolates across the host genotypes. Downy mildew incidence ranged from 0.66 per cent on IP 18297 with the isolate Sg 139-1 to 100 per cent on P 1564 with Sg 139-4 (Table 9). All isolates were highly aggressive on the susceptible genotype 7042S, relatively highly aggressive on IP 18292, P 1564, P 2895-3 and IP 5272-1 and moderately aggressive on 700481-21-8, while low to moderate aggressiveness was observed for all the isolates on P 536-3, IP 18296 and P 3281-1 and low aggressiveness on IP 18297. Among the isolates, Sg 139-4 recorded the highest mean disease incidence (64.29 %) across the host genotypes, followed by Sg 139-3 (61.68 %), while Sg 139-1 recorded the lowest (34.57 %) disease incidence (Table 9). The disease incidence induced by 10 SZIs on 10 host genotypes was quite variable and was significantly different ($P<0.01$) for various isolate-genotype combinations (Appendix V).

Table 9: Per cent disease incidence[a] of 10 single - zoospore isolates of Sg 139 on ten differential host genotypes

Isolates	Differential host genotypes										Mean
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 139-1	30 33	0 00	0 66	28 64	52 11	36 25	0 00	44 08	63 15	90 50	34 57
	(33 42)[b]	(0 00)	(4 65)	(32 35)	(46 21)	(37 02)	(0 00)	(41 60)	(52 62)	(72 05)	(31 99)
Sg 139-2	85 25	3 53	0 00	11 16	77 00	80 10	6 61	55 26	74 23	93 50	48 66
	(67 42)	(10 71)	(0 00)	(19 51)	(61 34)	(63 51)	(14 89)	(48 02)	(59 49)	(75 24)	(42 01)
Sg 139-3	94 87	23 65	1 97	46 50	93 74	95 33	0 82	69 25	94 21	96 50	61 68
	(76 91)	(29 10)	(8 06)	(43 00)	(75 51)	(77 59)	(5 18)	(56 32)	(76 09)	(79 24)	(52 70)
Sg 139-4	96 40	38 72	0 00	30 55	100 00	76 10	16 11	88 45	99 25	97 35	64 29
	(79 09)	(38 48)	(0 00)	(33 55)	(90 00)	(60 73)	(23 67)	(70 13)	(85 95)	(80 70)	(56 23)
Sg 139-5	51 16	13 91	1 43	9 15	75 13	88 57	10 50	78 46	89 06	91 27	50 86
	(45 67)	(21 90)	(6 86)	(17 61)	(60 09)	(70 24)	(18 91)	(62 35)	(70 68)	(72 83)	(44 71)
Sg 139-6	86 50	15 09	0 00	6 50	89 12	90 00	8 66	71 25	90 25	93 06	55 04
	(68 45)	(22 86)	(0 00)	(14 77)	(70 74)	(71 57)	(17 11)	(57 58)	(71 81)	(74 73)	(46 96)
Sg 139-7	86 97	7 98	0 00	9 56	58 29	54 04	3 51	50 30	56 95	89 15	41 67
	(68 84)	(16 41)	(0 00)	(18 01)	(49 77)	(47 32)	(10 79)	(45 17)	(49 00)	(70 77)	(37 61)
Sg 139-8	37 44	22 04	0 00	55 87	90 00	82 74	35 24	29 62	87 50	95 18	53 56
	(37 73)	(28 00)	(0 00)	(48 37)	(71 62)	(65 45)	(36 42)	(32 98)	(69 30)	(77 36)	(46 72)
Sg 139-9	85 28	1 55	0 00	5 37	81 07	66 45	20 16	48 25	92 75	95 43	49 63
	(67 44)	(7 15)	(0 00)	(13 39)	(64 21)	(54 60)	(26 68)	(44 00)	(74 38)	(77 66)	(42 95)
Sg 139-10	77 40	0 00	0 00	46 77	62 50	93 03	0 00	21 42	60 00	97 86	45 90
	(61 62)	(0 00)	(0 00)	(43 15)	(52 24)	(74 72)	(0 00)	(27 57)	(50 77)	(81 59)	(39 17)
Mean	73 16	12 65	0 41	25 01	77 90	76 26	10 16	55 63	80 73	93 98	
	(62 66)	(17 46)	(1 96)	(28 37)	(64 17)	(62 27)	(15 36)	(48 57)	(66 01)	(76 22)	

	S.Ed. ±	CD 1%
Isolates	0 1630	0 4198
Host genotypes	0 1630	0 4198
Interaction	0 5153	1 3275

[a] Mean of three replications

[b] Figures in parentheses are arcsin transformed values

4.1.3.3 Variation in disease reaction

Most of the isolate-genotype combinations yielded susceptible reaction although they had different levels of disease incidence (Table 10). All isolates showed susceptible reaction on host genotypes IP 5272-1, P 1564, P 2895-3, 700481-21-8, IP 18292 and 7042S, while the resistant reaction was observed for all isolates on IP 18297. Isolates had clear differential disease reactions on host genotypes IP 18296, P 536-2 and P 3281-1, but not on others (Table 10).

4.1.3.4 Variation in latent period

Latent period varied considerably for various isolate-genotype combinations and ranged between 5 to 10 days (Table 11). Significantly lowest latent period was observed for the isolate Sg 139-4 (6.72 days), and the greatest for Sg 139-7 (8.27 days) followed by Sg 139-9 (7.99 days), Sg 139-5 (7.98 days) and Sg 139-2 (7.94 days), and no significant difference was found between isolates Sg 139-9, Sg 139-5 and Sg 139-2. The lowest mean latent period (5.43 days) was recorded on the susceptible host genotype 7042S, and the longest mean latent period on P 3281-1 (8.78 days) and IP 18296 (8.73 days). However, mean sum of squares for isolates, host genotypes and their interaction were highly significant ($P<0.01$) (Appendix VI). A significant negative correlation ($r = -0.55$ at $P<0.01$) was found between latent period and disease incidence across the host genotypes and isolates.

4.1.3.5 Variation in virulence index

Significant differences were observed in virulence index of the isolates across the differential host genotypes (Table 12). Virulence index ranged from

Table 10: Disease reaction of ten single - zoospore isolates of Sg 139 on ten differential host genotypes

Isolates	Differential host genotypes									
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S
Sg 139-1	S	R	R	S	S	S	R	S	S	S
Sg 139-2	S	R	R	S	S	S	R	S	S	S
Sg 139-3	S	S	R	S	S	S	R	S	S	S
Sg 139-4	S	S	R	S	S	S	S	S	S	S
Sg 139-5	S	S	R	R	S	S	S	S	S	S
Sg 139-6	S	S	R	R	S	S	R	S	S	S
Sg 139-7	S	R	R	R	S	S	R	S	S	S
Sg 139-8	S	S	R	S	S	S	S	S	S	S
Sg 139-9	S	R	R	R	S	S	S	S	S	S
Sg 139-10	S	R	R	S	S	S	R	S	S	S

R Resistant reaction (<10% disease incidence)

S Susceptible reaction (≥10% disease incidence)

Table 11: Latent period[a] of ten single - zoospore isolates of Sg 139 on ten differential host genotypes

Isolates	Differential host genotypes									
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S
Sg 139-1	9 22	-[b]	7 33	8 44	6 67	8 00	-	8 67	8 16	5 28	7 73
Sg 139-2	8 16	9 67	-	9 00	7 33	7 00	9 60	8 33	7 05	5 33	7 94
Sg 139-3	6 50	8 33	7 72	7 72	6 39	6 05	10 00	7 22	6 16	5 33	7 14
Sg 139-4	5 67	7 61	-	8 33	5 83	7 61	8 05	6 44	6 00	5 00	6 72
Sg 139-5	8 83	8 33	9 05	9 50	8 00	7 44	8 22	7 50	7 16	5 78	7 98
Sg 139-6	8 05	8 00	-	8 56	7 16	7 33	8 67	7 16	7 50	5 61	7 56
Sg 139-7	6 50	9 61	-	9 83	8 05	8 50	9 83	7 33	8 78	6 00	8 27
Sg 139-8	8 00	8 78	-	7 00	6 22	8 50	7 33	8 72	7 61	5 61	7 53
Sg 139-9	8 16	9 50	-	9 00	7 50	8 00	8 50	7 89	8 05	5 33	7 99
Sg 139-10	8 33	-	-	7 50	8 00	7 00	-	8 83	8 00	5 00	7 53
Mean	7 74	8 73	8 03	8 49	7 11	7 55	8 78	7 81	7 45	5 43	

	S.Ed. ±	CD 1%
Isolates	0 0345	0 0889
Host genotypes	0 0345	0 0889
Interaction	0 2139	0 2811

[a] Mean of three replications

[b] No symptoms observed

Table 12: Virulence Index[a] of ten single - zoospore isolates of Sg 139 on ten differential host genotypes

Isolates	Differential host genotypes										Mean
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 139-1	3 29	0 00	0 09	3 39	7 82	4 53	0 00	5 08	7 74	17 18	4 91
Sg 139-2	10 45	0 36	0 00	1 24	10 51	11 44	0 69	6 63	10 53	17 55	6 94
Sg 139-3	14 60	2 84	0 26	6 02	14 69	15 75	0 08	9 60	15 30	18 12	9 73
Sg 139-4	17 02	5 09	0 00	3 67	17 15	10 00	2 00	13 73	16 54	19 47	10 47
Sg 139-5	5 79	1 67	0 16	0 96	9 39	11 90	1 28	10 46	12 44	15 81	6 99
Sg 139-6	10 74	1 89	0 00	0 76	12 45	12 28	1 00	9 95	12 03	16 58	7 77
Sg 139-7	13 38	0 83	0 00	0 97	7 24	6 36	0 35	6 87	6 49	14 86	5 73
Sg 139-8	4 68	2 51	0 00	7 98	14 48	9 74	4 81	3 40	11 49	17 00	7 61
Sg 139-9	10 45	0 16	0 00	0 60	10 81	8 31	2 37	6 12	11 52	17 91	6 83
Sg 139-10	9 30	0 00	0 00	6 25	7 81	13 29	0 00	2 43	7 50	19 57	6 62
Mean	9 97	1 54	0 05	3 19	11 24	10 36	1 26	7 43	11 16	17 40	

	S.Ed. ±	CD 1%
Isolates	0 0586	0 1511
Host genotypes	0 1150	0 1511
Interaction	0 3635	0 4778

[a] *Mean of three replications*

0.16 for isolate Sg 139-5 (on IP 18297) and for Sg 139-9 (on IP 18296) to 19.57 for Sg 139-10 (on 7042S). Among the isolates, Sg 139-4 had the highest virulence index (10.47) and the isolate Sg 139-1 had the lowest virulence index (4.91). In general, all isolates were found highly virulent on 7042S (17.40), followed by IP 18292 (11.16) and P 1564 (11.24) and least virulent on IP 18297 (0.05). No significant difference was found for mean virulence index between host genotypes IP 18292 and P 1564. Analysis of variance indicated highly significant ($P<0.01$) effects of isolates, host genotypes and their interaction on virulence index (Appendix VII).

4.1.3.6 Pathotype grouping

A dendrogram developed from the average linkage cluster analysis based on mean per cent disease incidence classified the 10 SZIs of Sg 139 at 83 per cent similarity level into five groups (Fig. 4). Isolates Sg 139-1 and Sg 139-7 formed group I; Sg 139-10 group II; Sg 139-2, Sg 139-6, Sg 139-9 and Sg 139-5 group III; Sg 139-3 and Sg 139-4 group IV and Sg 139-8 group V.

4.1.4 Evaluation of single-zoospore isolates (SZIs) of Sg 110, a weakly virulent parental isolate for pathogenicity on host differentials

The data on per cent disease incidence, disease reaction, latent period and virulence index of SZIs derived from a weakly virulent parental isolate, Sg 110 are presented in Tables 13-16.

4.1.4.1 Variation in virulence

All isolates on host genotypes IP 18296 and IP 18292, and majority of the isolates on IP 18297 and P 3281-1, with few exceptions, failed to cause

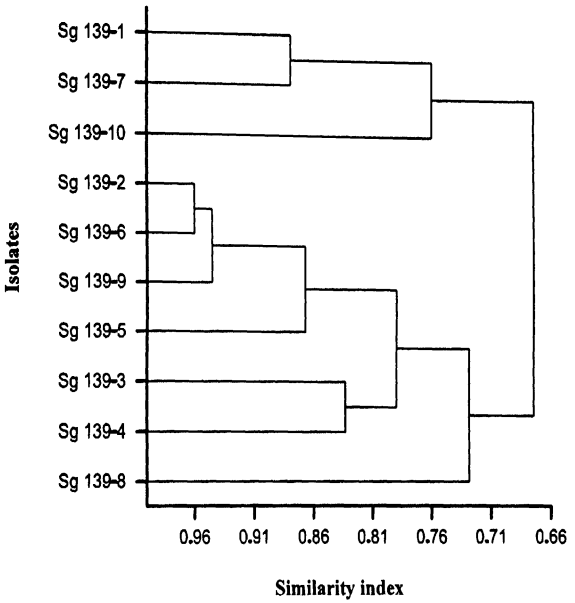

Fig.4: Dendrogram of 10 single-zoospore isolates of Sg 139 based on cluster analysis of disease incidence recorded on 10 differential host genotypes

infection and thus were considered avirulent, while all the isolates were virulent on the susceptible host genotype 7042S (Table 13). Isolates Sg 110-5 and Sg 110-3 with low disease incidence were virulent on IP 18297 and P 3281-1 respectively. However, the remaining isolate-genotype combinations showed virulent reaction (Table 13).

4.1.4.2 Variation in aggressiveness

The aggressiveness of the 10 SZIs of Sg 110 varied significantly across the host genotypes with disease incidence ranging between 0.34 and 96.42 per cent (Table 13). All the isolates were highly aggressive on the susceptible host genotype 7042S, while most of the isolates showed low to moderate aggressiveness on genotypes IP 5272-1, P 536-2 and 700481-21-8, and low aggressiveness on P 1564 and P 2895-3. Across the host genotypes, isolate Sg 110-3 recorded the highest mean disease incidence (25.71 %) and isolate Sg 110-9 the least (9.77 %) (Table 13). The effects of isolates, host genotypes and their interaction were highly significant ($P<0.01$) for disease incidence (Appendix VIII).

4.1.4.3 Variation in disease reaction

All isolates showed resistant reaction on genotypes IP 18296, IP 18297, P 3281-1 and IP 18292 and susceptible reaction on 7042S, and the isolates could not be distinguished on these genotypes (Table 14). However, clear differential reactions were obtained on the remaining five host genotypes.

Table 13: Per cent disease incidence[a] of ten single - zoospore isolates of Sg 110 on ten differential host genotype

Isolates	Differential host genotypes										Mean
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 110-1	3 40	0 00	0 00	25 99	7 33	8 46	0 00	22 46	0 00	93 06	16 07
	(10 63)[b]	(0 00)	(0 00)	(30 65)	(15 71)	(16 91)	(0 00)	(28 29)	(0 00)	(74 72)	(17 69)
Sg 110-2	0 00	0 00	0 00	5 37	0 00	0 00	0 00	13 51	0 00	88 71	10 76
	(0 00)	(0 00)	(0 00)	(13 39)	(0 00)	(0 00)	(0 00)	(21 56)	(0 00)	(70 37)	(10 53)
Sg 110-3	40 75	0 00	0 00	62 41	14 63	0 00	0 67	43 14	0 00	95 49	25 71
	(39 67)	(0 00)	(0 00)	(52 19)	(22 49)	(0 00)	(4 69)	(41 06)	(0 00)	(77 76)	(23 78)
Sg 110-4	0 00	0 00	0 00	22 50	2 55	0 00	0 00	8 31	0 00	94 66	12 80
	(0 00)	(0 00)	(0 00)	(28 32)	(9 19)	(0 00)	(0 00)	(16 75)	(0 00)	(76 65)	(13 09)
Sg 110-5	37 00	0 00	2 63	30 75	3 52	4 07	0 00	31 14	0 00	94 97	20 41
	(37 47)	(0 00)	(9 34	(33 68)	(10 81)	(11 63)	(0 00)	(33 92)	(0 00)	(77 04)	(21 39)
Sg 110-6	6 33	0 00	0 00	18 25	0 00	11 00	0 00	26 44	0 00	89 72	15 17
	(14 57)	(0 00)	(0 00)	(25 29)	(0 00)	(19 37)	(0 00)	(30 95)	(0 00)	(71 30)	(16 15)
Sg 110-7	13 08	0 00	0 00	45 00	2 29	26 12	0 00	8 50	0 00	93 83	18 88
	(21 20)	(0 00)	(0 00)	(42 13)	(8 70)	(30 73)	(0 00)	(16 95)	(0 00)	(75 61)	(19 53)
Sg 110-8	22 87	0 00	0 00	26 35	1 57	5 63	0 00	20 99	0 00	93 29	17 07
	(28 57)	(0 00)	(0 00)	(30 89)	(7 20)	(13 73)	(0 00)	(27 27)	(0 00)	(74 98)	(18 26)
Sg 110-9	0 00	0 00	0 00	0 34	0 00	0 00	0 00	5 56	0 00	91 76	9 77
	(0 00)	(0 00)	(0 00)	(3 32)	(0 00)	(0 00)	(0 00)	(13 63)	(0 00)	(73 32)	(9 03)
Sg 110-10	1 55	0 00	0 00	28 12	0 00	0 00	0 00	0 83	0 00	96 42	12 69
	(7 16)	(0 00)	(0 00)	(32 02)	(0 00)	(0 00)	(0 00)	(5 17)	(0 00)	(79 09)	(12 34)
Mean	12 50	0 00	0 26	26 51	3 19	5 53	0 07	18 09	0 00	93 19	
	(15 93)	(0 00)	(0 93)	(29 19)	(7 41)	(9 24)	(0 47)	(23 55)	(0 00)	(75 08)	

	S.Ed. ±	CD 1%
Isolates	0 0523	0 1347
Host genotypes	0 0523	0 1347
Interaction	0 1654	0 4260

[a] Mean of three replications

[b] Figures in parentheses are arcsin transformed values

Table 14: Disease reaction of ten single - zoospore isolates of Sg 110 on ten differential host genotypes

Isolates	Differential host genotypes									
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042 S
Sg 110-1	R	R	R	S	S	R	R	S	R	S
Sg 110-2	R	R	R	R	R	R	R	S	R	S
Sg 110-3	S	R	R	S	S	R	R	S	R	S
Sg 110-4	R	R	R	S	R	R	R	R	R	S
Sg 110-5	S	R	R	S	R	R	R	S	R	S
Sg 110-6	R	R	R	S	R	S	R	S	R	S
Sg 110-7	S	R	R	S	R	S	R	R	R	S
Sg 110-8	S	R	R	S	R	R	R	S	R	S
Sg 110-9	R	R	R	R	R	R	R	R	R	S
Sg 110-10	R	R	R	S	R	R	R	R	R	S

R : Resistant reaction (<10% disease incidence)

S : Susceptible reaction (³10% disease incidence)

4.1.4.4 Variation in latent period

The single-zoospore isolates of Sg 110 varied significantly for latent period ranging from 5.33 to 10.75 days (Table 15). The maximum mean latent period was observed on host genotype P 3281-1 (10.00 days), followed by P 1564 (9.63 days), while that of the minimum was observed on the susceptible genotype 7042S (5.79 days). Across the host genotypes, isolate Sg 110-3 had the shortest latent period (8.00 days), while the longest was observed for isolate Sg 110-7 (8.96 days) and 110-4 (8.92 days), and there was no significant difference between Sg 110-7 and Sg 110-4 (Table 15). Highly significant ($P<0.01$) effects of isolates, host genotypes and their interaction on latent period were observed (Appendix IX). A significant negative correlation ($r = -0.92$ at $P<0.01$) was found between latent period and disease incidence across host genotypes and isolates.

4.1.4.5 Variation in virulence index

Significant differences were observed in virulence index of the isolates across the host differentials and it ranged from 0.03 to 17.93 (Table 16). Among the isolates, Sg 110-3 had the highest mean virulence index (3.85), while Sg 110-9 had the lowest (1.57) across the host genotypes. Among the host genotypes, the highest virulence index was recorded on 7042S (16.17), followed by P 536-2 (3.18) and the lowest on P 3282-1 (0.01). The virulence index was highly influenced by isolates, host lines and their interaction (Appendix X).

Table 15: Latent period[a] of ten single - zoospore isolates of Sg 110 on ten differential host genotypes

Isolates	Differential host genotypes									
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S
Sg 110-1	9 40	-	-	8 72	9 05	8 39	-	8 83	-	5 50	8 32
Sg 110-2	-[b]	-	-	10 00	-	-	-	9 50	-	5 89	8 46
Sg 110-3	7 33	-	-	7 50	9 16	-	10 00	8 67	-	5 33	8 00
Sg 110-4	-	-	-	9 00	10 50	-	-	10 00	-	6 16	8 92
Sg 110-5	7 22	-	8 00	8 78	9 33	8 83	-	8 50	-	5 50	8 02
Sg 110-6	8 67	-	-	8 00	-	10 50	-	8 83	-	5 67	8 33
Sg 110-7	9 83	-	-	8 72	10 48	9 05	-	9 44	-	6 22	8 96
Sg 110-8	8 17	-	-	8 66	9 25	9 33	-	7 83	-	5 56	8 13
Sg 110-9	-	-	-	10 00	-	-	-	10 50	-	6 05	8 85
Sg 110-10	10 75	-	-	8 11	-	-	-	9 67	-	6 00	8 63
Mean	8 77	-	8 00	8 75	9 63	9 22	10 00	9 18	-	5 79	

	S.Ed. ±	CD 1%
Isolates	0 0456	0 1174
Host genotypes	0 0893	0 1174
Interaction	0 2825	0 3713

[a] Mean of three replications

[b] No symptoms observed

Table 16: Virulence index[a] of ten single - zoospore isolates of Sg 110 on ten differential host genotypes

Isolates	Differential host genotypes										Mean
	IP 5272-11	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 110-1	0 36	0 00	0 00	2 98	0 81	1 01	0 00	2 54	0 00	17 01	2 47
Sg 110-2	0 00	0 00	0 00	0 54	0 00	0 00	0 00	1 42	0 00	15 07	1 70
Sg 110-3	5 56	0 00	0 00	8 32	1 60	0 00	0 07	4 98	0 00	17 93	3 85
Sg 110-4	0 00	0 00	0 00	2 50	0 24	0 00	0 00	0 83	0 00	15 37	1 90
Sg 110-5	5 13	0 00	0 33	3 50	0 38	0 46	0 00	3 67	0 00	17 36	3 08
Sg 110-6	0 73	0 00	0 00	2 29	0 00	1 05	0 00	2 99	0 00	15 84	2 29
Sg 110-7	1 33	0 00	0 00	5 16	0 22	2 88	0 00	0 90	0 00	15 09	2 56
Sg 110-8	2 80	0 00	0 00	3 04	0 17	0 61	0 00	2 68	0 00	16 79	2 61
Sg 110-9	0 00	0 00	0 00	0 03	0 00	0 00	0 00	0 53	0 00	15 16	1 57
Sg 110-10	0 15	0 00	0 00	3 47	0 00	0 00	0 00	0 08	0 00	16 07	1 98
Mean	1 61	0 00	0 03	3 18	0 34	0 60	0 01	2 06	0 00	16 17	

	S.Ed. ±	CD 1%
Isolates	0 0646	0 1663
Host genotypes	0 1266	0 1663
Interaction	0 4002	0 5260

[a] Mean of three replications

4.1.4.6 Pathotype grouping

A dendrogram developed from the average linkage cluster analysis based on mean per cent disease incidence classified the 10 SZIs of Sg 110 at 90 per cent similarity level into four groups (Fig. 5). Isolates Sg 110-1, Sg 110-6, Sg 110-8, Sg 110-2, Sg 110-9, Sg 110-4 and Sg 110-10 were in group I; isolates Sg 110-7, Sg 110-3 and Sg 110-5 were not clustered at the 90 per cent similarity level and formed separate groups, group II, group III and group IV, respectively.

4.2 ASSESSMENT OF GENETIC VARIABILITY AMONG PARENTAL ISOLATES OF *S. graminicola* USING AFLP MARKERS

A high level of polymorphism was obtained with AFLP analysis using three primer combinations (E-TG/M-CAT, E-TT/M-TAG and E-TG/M-CTA) among the 20 parental isolates of *S. graminicola*. (Due to some unknown reason, the genomic DNA of isolate Sg 032 did not give any fingerprint pattern on autoradiograms and therefore, it was eliminated while counting the bands for calculation of similarity index). The details of number of bands observed and number of polymorphic and monomorphic bands is presented in Table 17. A total of 185 bands were scored for the three primer combinations, of which about 80 per cent was found polymorphic.

With the primer combination E-TG/M-CAT, the bands designated 'a' and 'd' were specific to isolate Sg 026 and bands designated 'c' and 'e' were specific to isolate Sg 151, while the bands designated 'b', 'f' and 'g', which were found in all isolates were absent in isolates Sg 025, Sg 139 and Sg 151, respectively (Plate 3). Likewise, with the primer combination E-TT/M-TAG,

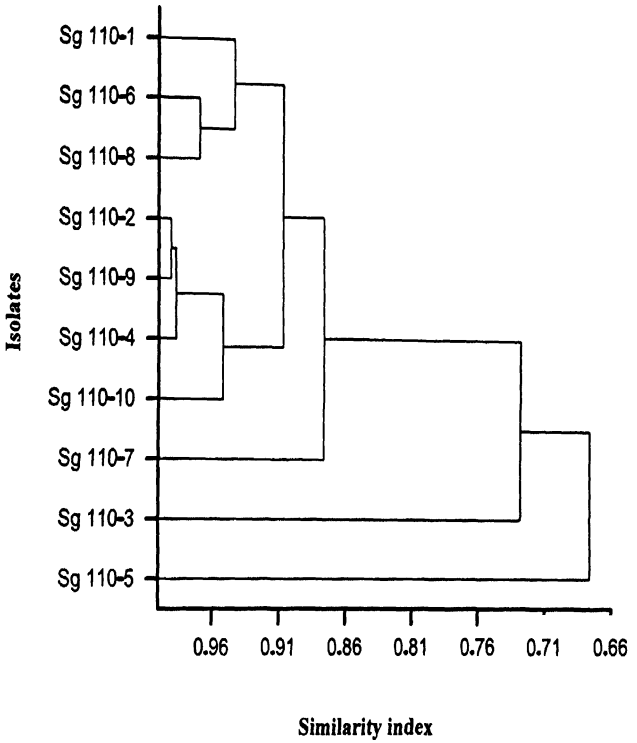

Fig.5: Dendrogram of 10 single-zoospore isolates of Sg 110 based on cluster analysis of disease incidence recorded on differential host genotypes

Table 17: Number of bands observed and number of polymorphic and monomorphic bands obtained in three primer combinations of AFLP analysis

Primer combination	Number of bands observed	Number of polymorphic bands	Number of monomorphic bands
E-TG/M-CAT	58	49	9
E-TT/M-TAG	63	51	12
E-TG/M-CTA	64	49	15
Total	185	149	36

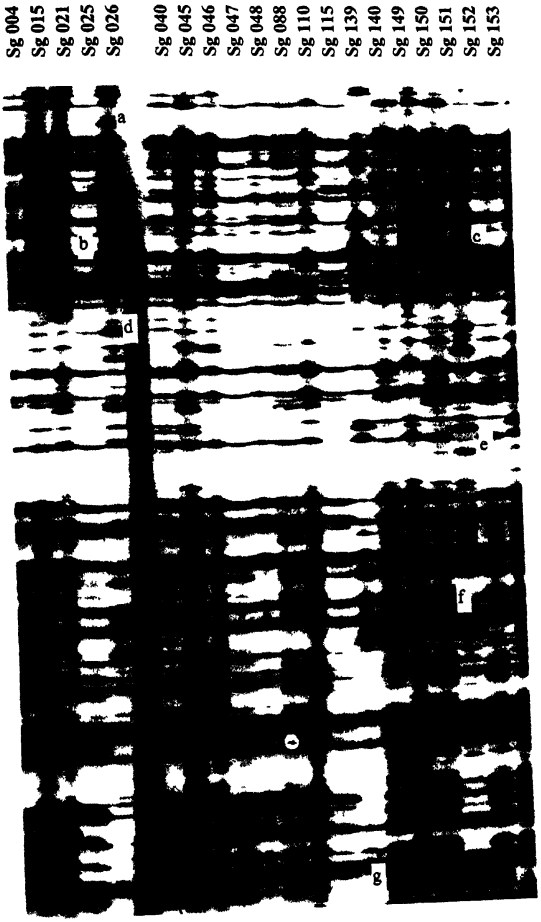

Plate 3: An autoradiogram of AFLP profiles of 20 parental isolates of *Sclerospora graminicola* using the primer combination E-TG/ M-CAT

Note: a, c, d and e = specific bands present, b, f and g = specific bands absent

the bands designated 'a', b' and 'g' were specific to isolate Sg 004 and the band designated 'e' was specific to isolate Sg 151, whereas the bands designated 'c', 'd' and 'f' found in all the isolates were absent in isolates Sg 026, Sg 139 and Sg 151, respectively (Plate 4). Similarly, with the primer combination E-TG/M-CTA, the bands designated 'a' and 'd' were specific to isolates Sg 026 and the band designated 'e' was specific to isolate Sg 139, while the bands designated 'b' and 'c' found in all the isolates were absent in isolate Sg 139 (Plate 5).

Cluster analysis of the similarity index data classified the 20 isolates into eight groups at 70 per cent similarity level and the classification is presented as dendrogram (Fig. 6). Isolates Sg 015, Sg 046, Sg 047, Sg 149, Sg 045, Sg 048, Sg 088 and Sg 040 were in group I; Sg 021, Sg 115 and Sg 153 in group II; Sg 110, Sg 150, Sg 152, and Sg 140 in group III. The isolates Sg 004, Sg 026, Sg 025, Sg 139 and Sg 151 were not clustered at the 70 per cent similarity level and thus formed separate groups, group IV, V, VI, VII and VIII, respectively.

4.3 IDENTIFICATION OF MATING TYPES AMONG THE ISOLATES OF *S.graminicola*

4.3.1 Determination of mating types

Of the 70 SZIs when inoculated alone, no oospores were produced in 62 isolates and oospores in fewer numbers (<50 oospores/cm^2 leaf area) were observed in 8 isolates. The oospore production per unit leaf area in these 8 isolates is given in Table 18. Isolates in which oospore production was not

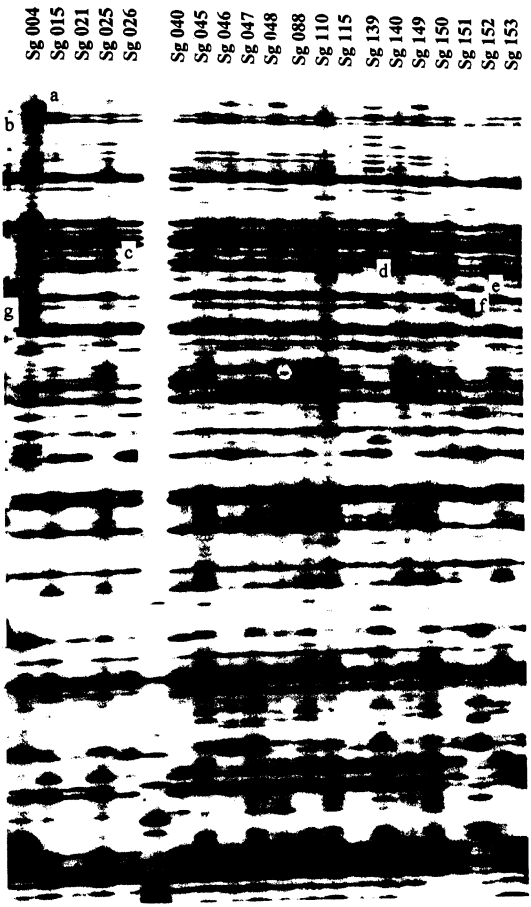

Plate 4: An autoradiogram of AFLP profiles of 20 parental isolates of *Sclerospora graminicola* using the primer combination E-TT/M-TAG

Note: a, b, e and g = specific bands present; c, d and f = specific bands absent

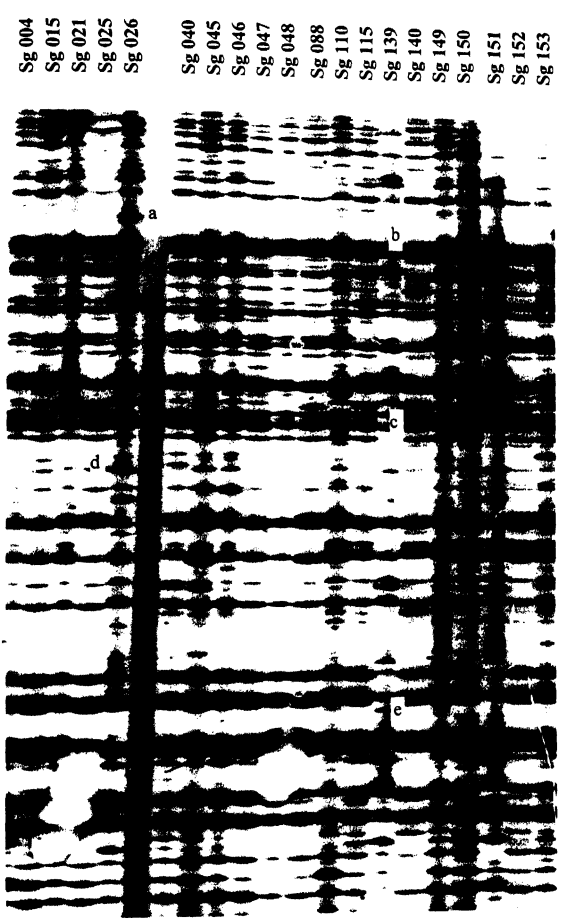

Plate 5: An autoradiogram of AFLP profiles of 20 parental isolates of *Sclerospora graminicola* using the primer combination E-TG/M-CTA

Note: a, d and e = specific bands present; b and c = specific bands absent

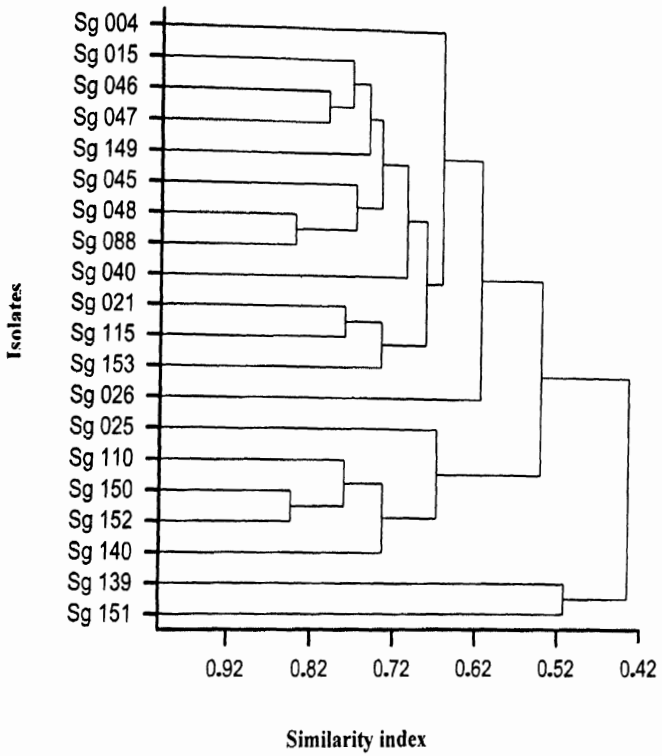

Fig.6: Dendrogram of 20 parental isolates of *Sclerospora graminicola* based on cluster analysis of AFLP data with three primer combinations

Table 18 : Oospore production in eight self-fertile single-zoospore isolates when inoculated alone

S.No.	Single-zoospore isolates	No. of oospores produced in unit leaf area[a]
1	Sg 021-5	15
2	Sg 021-8	46
3	Sg 021-9	14
4	Sg 149-8	22
5	Sg 110-3	13
6	Sg 110-5	25
7	Sg 110-6	31
8	Sg 110-8	18

[a]: Mean of 20 leaf samples

observed were referred as self-sterile isolates and those supported oospore production referred as self-fertile isolates.

The 16 self-sterile SZIs used to determine mating types fell into two groups, when inoculated in all possible paired combinations (Table 19). Isolates Sg 139-1, Sg 139-3, Sg 139-5, Sg 139-6, Sg 139-7, Sg 139-10 and Sg 110-9 belonged to one group and isolates Sg 139-2, Sg 139-4, Sg 139-8, Sg 139-9, Sg 110-1, Sg 110-4, Sg 110-7 and Sg 110-10 belonged to the other group (Table 19). Oospores were produced in abundance when any one of the isolates of one group was paired with any one of the isolates of the other group and vice versa. Inoculations with isolates of the same group in all paired combinations did not result in the production of oospores. The isolate Sg 110-2 failed to produce oospores with any one of the isolates tested and behaved unique of its kind. The two mating type groups identified were designated, Mat A and Mat B.

4.3.2 Composition, frequency and distribution of mating types

To know the composition, frequency and distribution of mating types among the isolates of *S. graminicola*, the 70 SZIs derived from the seven parental isolates were tested for oospore production by pairing with the two standard mating type isolates PT 2 and PT 3 and the results obtained are presented in table 30. Arbitrarily, the isolates that produced oospores with the isolate PT 3 were placed in group Mat A (PT2 type) whereas those that produced oospores with the isolate PT 2 were placed in group Mat B (PT 3 type). Oospore production was abundant in matings involving self-sterile isolates whereas it was found sparse in matings involving self-fertile isolates (Table 20).

Table 19 : Mating type behaviour of 16 single-zoospore isolates of *Sclerospora graminicola*

	Sg 110-10	Sg 110-9	Sg 110-7	Sg 110-4	Sg 110-2	Sg 110-1	Sg 139-10	Sg 139-9	Sg 139-8	Sg 139-7	Sg 139-6	Sg 139-5	Sg 139-4	Sg 139-3	Sg 139-2	Sg 139-1
Sg 139-1	+	-	+	+	-	+	-	+	+	-	-	-	+	-	+	-
Sg 139-2	-	+	-	-	-	-	+	-	-	+	+	+	-	+	-	
Sg 139-3	+	-	+	+	-	+	-	+	+	-	-	-	+	-		
Sg 139-4	-	+	-	-	-	-	+	-	-	+	+	+	-			
Sg 139-5	+	-	+	+	-	+	-	+	+	-	-	-				
Sg 139-6	+	-	+	+	-	+	-	+	+	-	-					
Sg 139-7	+	-	+	+	-	+	-	+	+	-						
Sg 139-8	-		-	-	-	-	+	-	-							
Sg 139-9	-	+	-	-	-	-	+	-								
Sg 139-10	+	-	+	+	-	+	-									
Sg 110-1	-	+	-	-	-	-										
Sg 110-2	-	-	-	-	-											
Sg 110-4	-	+	-	-												
Sg 110-7	-	+	-													
Sg 110-9	+	-														
Sg 110-10	-															

+ : Abundant oospore production
- : No oospore production

Table 20 : Oospore production in 70 single-zoospore isolates when paired with the two standard mating type isolates PT2 and PT3

S.No.	Single- zoospore isolates	Oospore production[a] when paired with	
		PT2	PT3
1	Sg 048-1	>1000[c]	-[b]
2	Sg 048-2	>1000	-
3	Sg 048-3	-	>1000
4	Sg 048-4	>1000	-
5	Sg 048-5	862	-
6	Sg 048-6	>1000	-
7	Sg 048-7	-	>1000
8	Sg 048-8	-	>1000
9	Sg 048-9	740	-
10	Sg 048-10	-	>1000
11	Sg 021-1	-	954
12	Sg 021-2	-	>1000
13	Sg 021-3	>1000	-
14	Sg 021-4	>1000	-
15	Sg 021-5*	460	215
16	Sg 021-6	-	>1000
17	Sg 021-7	>1000	-
18	Sg 021-8*	522	186
19	Sg 021-9*	175	441
20	Sg 021-10	>1000	-
21	Sg 149-1	-	921
22	Sg 149-2	-	>1000
23	Sg 149-3	-	>1000
24	Sg 149-4	-	>1000
25	Sg 149-5	>1000	-
26	Sg 149-6	-	>1000
27	Sg 149-7	>1000	-
28	Sg 149-8*	213	584
29	Sg 149-9	-	>1000
30	Sg 149-10	>1000	-
31	Sg 110-1	-	>1000
32	Sg 110-2	-	-
33	Sg 110-3*	432	432
34	Sg 110-4	-	833
35	Sg 110-5*	126	472
36	Sg 110-6*	137	556
37	Sg 110-7	-	>1000
38	Sg 110-8*	396	487
39	Sg 110-9	>1000	-
40	Sg 110-10	-	>1000

Contd.

Contd...

S.No.	Single- zoospore isolates	Oospore production when paired with	
		PT2	PT3
41	Sg 153-1	-	>1000
42	Sg 153-2	-	>1000
43	Sg 153-3	-	>1000
44	Sg 153-4	>1000	-
45	Sg 153-5	>1000	-
46	Sg 153-6	-	903
47	Sg 153-7	>1000	-
48	Sg 153-8	-	>1000
49	Sg 153-9	>1000	-
50	Sg 153-10	>1000	-
51	Sg 139-1	>1000	-
52	Sg 139-2	-	>1000
53	Sg 139-3	>1000	-
54	Sg 139-4	-	>1000
55	Sg 139-5	>1000	-
56	Sg 139-6	>1000	-
57	Sg 139-7	>1000	-
58	Sg 139-8	-	>1000
59	Sg 139-9	-	>1000
60	Sg 139-10	>1000	-
61	Sg 152-1	-	>1000
62	Sg 152-2	>1000	-
63	Sg 152-3	>1000	-
64	Sg 152-4	782	-
65	Sg 152-5	-	>1000
66	Sg 152-6	>1000	-
67	Sg 152-7	>1000	-
68	Sg 152-8	>1000	-
69	Sg 152-9	>1000	-
70	Sg 152-10	>1000	-

[a] : Mean of 20 leaf samples

[b] : No oospores

[c] : Numerous oospores

* : Self-fertile isolates

Of the 70 SZIs analysed 28 (40.00%) behaved as Mat A, 33 (47.14%) as Mat B and 8 (11.43%) behaved as both Mat A and Mat B, while 1 (1.43%) behaved differently without producing oospores with either of the mating type isolates (Table 21). The 28 isolates, which produced oospores only with PT3 and the 33 isolates, which produced oospores only with PT 2 were self-sterile and the 8 isolates which produced oospores with both these isolates were self-fertile. Among the 8 self-fertile isolates, 5 produced more oospores with the isolate PT 3 than with the isolate PT 2, two isolates produced more oospores with isolate PT 2 than with PT 3, and one isolate produced oospores equally with both the isolates.

The ratio 28 Mat A: 33 Mat B do not differ significantly from a 1:1 ratio (χ^2 1:1 = 0.40, $P > 0.01$) and the results suggests the occurrence of Mat A and Mat B mating types in equal frequencies among the seven selected parental isolates of *S. graminicola*. Though the overall frequencies of both the mating types was found to be in equal proportions, their distribution within the individual parental isolates was not uniform, especially in isolates Sg 149 (6 Mat A : 3 Mat B), Sg 110 (4 Mat A : 1 Mat B) and Sg 152 (2 Mat A :8 Mat B). In these isolates, the frequency of occurrence of one mating type was found higher than the other (Table 21).

4.4 INHERITANCE OF VIRULENCE IN *S. graminicola*

The F_1, F_2, BC_1 and BC_2 progeny isolates were evaluated for virulence phenotype on the tester host genotype IP 18292 and the results obtained are presented in Tables 22 and 23. All the 33 F_1 progeny isolates produced as a

Table 21 : Composition, frequency and distribution of mating types within and between the isolates of *Sclerospora graminicola*

Parental isolates	Mat A only	Mat B only	Mat A > Mat B	Mat B > Mat A	Mat A = Mat B	None of these	Total
Sg 048	4	6	0	0	0	0	10
Sg 149	6	3	1	0	0	0	10
Sg 021	3	4	1	2	0	0	10
Sg 110	4	1	3	0	1	1	10
Sg 153	5	5	0	0	0	0	10
Sg 139	4	6	0	0	0	0	10
Sg 152	2	8	0	0	0	0	10
Total	28	33	5	2	1	1	70

Mat A only : Produced oospores only with PT3
Mat B only : Produced oospores only with PT2
} **Self-sterile isolates**

Mat A> Mat B : Produced more oospores with the isolate PT3 than with the isolate PT2
Mat B>Mat A : Produced more oospores with the isolate PT2 than with the isolate PT3
Mat A=Mat B : Produced oospores equally with both the isolates
} **Self-fertile isolates**

None of these : No oospore production with either of the isolates - **Self-sterile isolate**

result of hybridization between the highly virulent parent, Sg 139-4 and the avirulent parent, Sg 110-9 showed avirulent reaction (-), and no segregation was observed (Table 22). Among the 230 F_2 progeny isolates evaluated, 177 were found avirulent (-) and 53 virulent (+), and a segregation ratio of 177:53 did not differ significantly from the ratio 3:1 (χ^2 3:1 = 0.46, P> 0.01) (Table 23). This segregation pattern of F_2 generation was supported by the segregation ratios obtained in BC_1 and BC_2 generations. The 46 backcross progeny isolates obtained from the backcross of F_1 with the avirulent parent (Sg 110-9) did not segregate and entire progeny was avirulent (Table 22). While, backcross of the F_1 with the virulent parent (Sg 130-4) segregated in the ratio, 28 avirulent : 34 virulent and the ratio of 28:34 is a good fit to a 1:1 ratio (χ^2 1:1 = 0.58, P>0.01) (Table 23). A schematic representation of inheritance of virulence study in *S. graminicola* is shown in Figure 7.

It is evident from the results (Tables 22 and 23) that the specific virulence in pathogen isolates Sg 139-4 and Sg 110-9 to match the resistance in IP 18292, seems to be determined by a pair of alleles (*A/a*) at a single locus with avirulence dominant to virulence. The segregation pattern would also suggest that, the parents are homozygous and F_1s are heterozygous at this locus. Based on data obtained (Table 22), the avirulent parent Sg 110-9 and the virulent parent Sg 139-4 are assumed to have homozygous dominant alleles for avirulence (*AA*) and homozygous recessive alleles for virulence (*aa*), respectively, to the tester host genotype IP 18292. The assumed genotypes of parents, F_1, F_2, BC_1 and BC_2 generation isolates are given in Table 23.

Table 22 : Virulence phenotypes of F_1, F_2, BC_1 and BC_2 progeny isolates of *Sclerospora graminicola* on the tester host genotype IP 18292

Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenoty
F_1 generation																			
F_1-1	-	F_1-2	-	F_1-3	-	F_1-4	-	F_1-5	-	F_1-6	-	F_1-7	-	F_1-8	-	F_1-9	-	F_1-10	-
F_1-11	-	F_1-12	-	F_1-13	-	F_1-14	-	F_1-15	-	F_1-16	-	F_1-17	-	F_1-18	-	F_1-19	-	F_1-20	-
F_1-21	-	F_1-22	-	F_1-23	-	F_1-24	-	F_1-25	-	F_1-26	-	F_1-27	-	F_1-28	-	F_1-29	-	F_1-30	-
F_1-31	-	F_1-32	-	F_1-33	-														
F_2 generation																			
F_2-1	-	F_2-2	-	F_2-3	-	F_2-4	+	F_2-5	-	F_2-6	-	F_2-7	-	F_2-8	-	F_2-9	-	F_2-10	-
F_2-11	+	F_2-12	-	F_2-13	+	F_2-14	-	F_2-15	-	F_2-16	+	F_2-17	-	F_2-18	+	F_2-19	-	F_2-20	-
F_2-21	+	F_2-22	-	F_2-23	+	F_2-24	-	F_2-25	+	F_2-26	+	F_2-27	-	F_2-28	-	F_2-29[a]	-	F_2-30	-
F_2-31	+	F_2-32	-	F_2-33	+	F_2-34	-	F_2-35	+	F_2-36	-	F_2-37	-	F_2-38	-	F_2-39	-	F_2-40	-
F_2-41	-	F_2-42	+	F_2-43	-	F_2-44	-	F_2-45	-	F_2-46	+	F_2-47	+	F_2-48	-	F_2-49	-	F_2-50	-
F_2-51[a]	-	F_2-52	-	F_2-53	-	F_2-54	+	F_2-55	-	F_2-56	-	F_2-57	-	F_2-58	+	F_2-59	-	F_2-60	-
F_2-61	-	F_2-62	-	F_2-63	-	F_2-64	+	F_2-65	-	F_2-66	-	F_2-67	+	F_2-68	-	F_2-69	-	F_2-70	-
F_2-71	-	F_2-72	-	F_2-73	-	F_2-74	+	F_2-75	-	F_2-76	+	F_2-77	+	F_2-78	-	F_2-79	-	F_2-80	-
F_2-81	-	F_2-82	-	F_2-83	-	F_2-84	+	F_2-85	-	F_2-86	-	F_2-87	-	F_2-88	-	F_2-89	-	F_2-90	-
F_2-91	-	F_2-92	-	F_2-93[a]	-	F_2-94	+	F_2-95	-	F_2-96	+	F_2-97	-	F_2-98	-	F_2-99	-	F_2-100	+
F_2-101	-	F_2-102	-	F_2-103	-	F_2-104	-	F_2-105	-	F_2-106	+	F_2-107	-	F_2-108	-	F_2-109	-	F_2-110	-
F_2-111	-	F_2-112	-	F_2-113	+	F_2-114	-	F_2-115	+	F_2-116	+	F_2-117	-	F_2-118	-	F_2-119	-	F_2-120	+
F_2-121	-	F_2-122	-	F_2-123	-	F_2-124	+	F_2-125	-	F_2-126	-	F_2-127	+	F_2-128	-	F_2-129	+	F_2-130	-
F_2-131	-	F_2-132	+	F_2-133	-	F_2-134	-	F_2-135	-	F_2-136	-	F_2-137	-	F_2-138[b]	-	F_2-139	-	F_2-140	-
F_2-141	-	F_2-142	+	F_2-143	+	F_2-144	-	F_2-145	-	F_2-146	-	F_2-147	-	F_2-148	-	F_2-149	+	F_2-150	-
F_2-151	-	F_2-152	-	F_2-153	-	F_2-154	-	F_2-155	-	F_2-156	-	F_2-157	-	F_2-158	+	F_2-159	-	F_2-160	-
F_2-161	-	F_2-162	-	F_2-163	+	F_2-164	-	F_2-165[a]	-	F_2-166	-	F_2-167	-	F_2-168	-	F_2-169	+	F_2-170	-
F_2-171	-	F_2-172	+	F_2-173	-	F_2-174	-	F_2-175	-	F_2-176	-	F_2-177	-	F_2-178	-	F_2-179	-	F_2-180	-
F_2-181	+	F_2-182	-	F_2-183	+	F_2-184[c]	-	F_2-185	-	F_2-186[a]	-	F_2-187	-	F_2-188	-	F_2-189	-	F_2-190	+
F_2-191	-	F_2-192	-	F_2-193	+	F_2-194	-	F_2-195	-	F_2-196	-	F_2-197	-	F_2-198	-	F_2-199	+	F_2-200	-
F_2-201	-	F_2-202	-	F_2-203	+	F_2-204	-	F_2-205	-	F_2-206	-	F_2-207	+	F_2-208	-	F_2-209	-	F_2-210	-
F_2-211	-	F_2-212	-	F_2-213	-	F_2-214	-	F_2-215	-	F_2-216	-	F_2-217	+	F_2-218	+	F_2-219	-	F_2-220	-
F_2-221	+	F_2-222	-	F_2-223	-	F_2-224	-	F_2-225	-	F_2-226	-	F_2-227	-	F_2-228	+	F_2-229	-	F_2-230	-

Contd.

Contd...

Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype	Isolate	Virulence phenotype
BC_1 generation																					
BC_1-1	-	BC_1-2	-	BC_1-3	-	BC_1-4	-	BC_1-5	-	BC_1-6	-	BC_1-7	-	BC_1-8	-	BC_1-9	-	BC_1-10	-		
BC_1-11	-	BC_1-12	-	BC_1-13	-	BC_1-14	-	BC_1-15	-	BC_1-16	-	BC_1-17	-	BC_1-18	-	BC_1-19	-	BC_1-20	-		
BC_1-21	-	BC_1-22	-	BC_1-23	-	BC_1-24	-	BC_1-25	-	BC_1-26	-	BC_1-27	-	BC_1-28	-	BC_1-29	-	BC_1-30	-		
BC_1-31	-	BC_1-32	-	BC_1-33	-	BC_1-34	-	BC_1-35	-	BC_1-36	-	BC_1-37	-	BC_1-38	-	BC_1-39	-	BC_1-40	-		
BC_1-41	-	BC_1-42	-	BC_1-43	-	BC_1-44	-	BC_1-45	-	BC_1-46	-										
BC_2 generation																					
BC_2-1	+	BC_2-2	-	BC_2-3	+	BC_2-4	+	BC_2-5	-	BC_2-6[b]	-	BC_2-7	+	BC_2-8	-	BC_2-9	+	BC_2-10	+		
BC_2-11	-	BC_2-12	+	BC_2-13	-	BC_2-14	+	BC_2-15	+	BC_2-16	+	BC_2-17	-	BC_2-18	-	BC_2-19	+	BC_2-20	+		
BC_2-21	+	BC_2-22	-	BC_2-23	-	BC_2-24	+	BC_2-25	-	BC_2-26	+	BC_2-27	+	BC_2-28	+	BC_2-29	+	BC_2-30	-		
BC_2-31	+	BC_2-32	+	BC_2-33	-	BC_2-34	-	BC_2-35	+	BC_2-36	-	BC_2-37	+	BC_2-38	-	BC_2-39	+	BC_2-40	-		
BC_2-41[a]	-	BC_2-42	+	BC_2-43	-	BC_2-44	-	BC_2-45	-	BC_2-46	+	BC_2-47	+	BC_2-48	+	BC_2-49	-	BC_2-50	-		
BC_2-51	+	BC_2-52	+	BC_2-53	+	BC_2-54	+	BC_2-55	-	BC_2-56	-	BC_2-57	+	BC_2-58	-	BC_2-59	-	BC_2-60	+		
BC_2-61	+	BC_2-62	-																		

+ : Virulence reaction
- : Avirulence reaction

a : One infected plant observed
b : Two infected plants observed
c : Three infected plants observed

Table 23 : **Observed and expected segregation of virulence phenotypes of F_1, F_2, BC_1 and BC_2 progeny isolates of *Sclerospora graminicola* together with assumed genotypes**

Isolates	Observed segregation	Expected segregation	Assumed genotypes		χ^2
	Avirulent : Virulent	Avirulent : Virulent	Avirulent	Virulent	
Sg 110-9 (Avirulent parent)	-	-	*AA*	-	-
Sg 139-4 (Virulent parent)	-	-	-	*aa*	-
F_1 progeny	33 : 0	1: 0	*Aa*	-	-
F_2 progeny	177 : 53	3 : 1	*AA & Aa*	*aa*	0.46
BC_1 progeny	46 : 0	1 : 0	*AA & Aa*	-	-
BC_2 progeny	28 : 34	1 : 1	*Aa*	*aa*	0.58

A : Dominant avirulent allele

a : Recessive virulent allele

χ^2 values are not significant ($P > 0.01$)

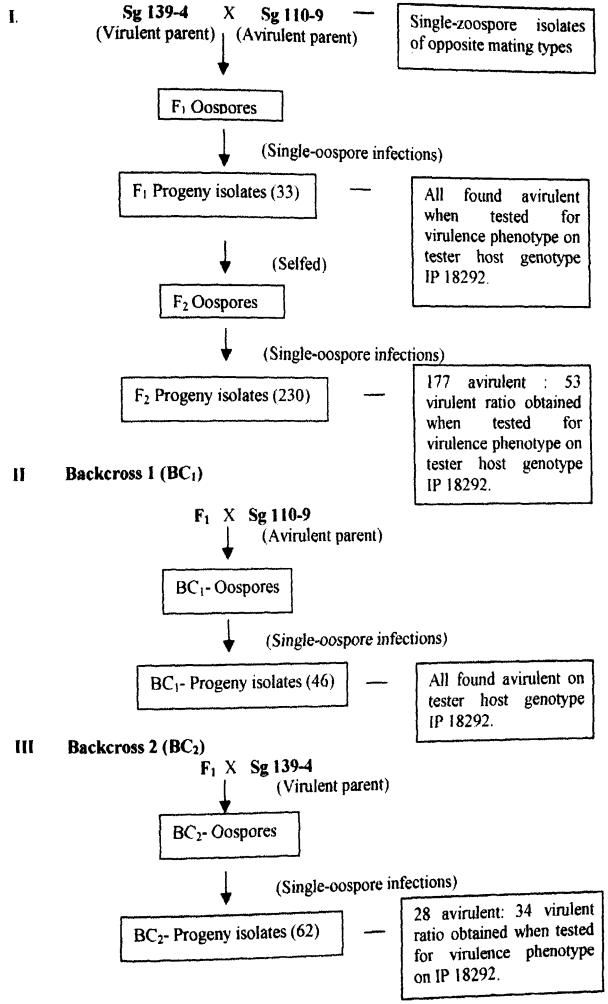

Fig.1 : Schematic representation of inheritance of virulence study in *Sclerospora. graminicola*

Discussion

CHAPTER-V

DISCUSSION

Results of the experiments conducted in the present investigation were critically examined and are discussed below.

5.1 CHARACTERIZATION OF ISOLATES OF *S. graminicola* FOR PATHOGENIC VARIABILITY

Variation among parental isolates

Significant variation in pathogenicity on a universally susceptible host genotype 7042S was recorded among the 21 parental isolates of *S. graminicola*. The highest disease incidence (76.15%) and shortest latent period (6.00 days) were recorded with the isolate Sg 153, and the lowest disease incidence (1.97% and 2.61%) and longest latent period (30 days) were recorded with the isolates Sg 021 and Sg 004 (Table 3). Generally, isolates collected during the crop season 1997 recorded significantly higher disease incidence than those collected in previous years and this could be attributed to the decreased viability and infectivity of oospores with the increasing shelf life. These results are in agreement with the earlier findings on survival, viability and infectivity of oospores of *S. graminicola* (Satyanarayana, 1963; Safeeulla, 1975; Thakur, 1981).

After the establishment of parental isolates, they were evaluated for pathogenicity on a set of ten differential host genotypes. The results indicated differential interactions for virulence, aggressiveness, disease reaction, latent period, virulence index and oospore production among the 21 parental isolates. Significant host X pathogen interaction (Appendix I) indicated existence of

specificity in the pearl millet-downy mildew pathosystem as hypothesised by Van der plank (1984). These results further support the findings on pathogenic variation among the sporangial (Thakur and Rao, 1997) and oospore (Thakur and Shetty, 1993) isolates of *S. graminicola*. All isolates were highly aggressive and virulent on the susceptible host genotype 7042S. However, majority of the isolates were avirulent on IP 18297; less aggressive on P 3281-1, IP 18296 and IP 18292; and moderately aggressive on 700481-21-8, P 2895-3, P 1564, P 536-2 and IP 5272-1 (Table 4) indicating the presence of a range of resistance genes in the host genotypes to the corresponding virulence genes in isolates. The host genotype IP 18292, which was included in the study as a resistant genotype, unexpectedly showed differential reaction to isolates evaluated with disease incidence ranging between zero and 89.55 per cent. This suggests the evolution of virulence factor(s) specific to host resistance factor(s) in pearl millet-downy mildew system. Evidences from this study and earlier findings (Appadurai *et al.*, 1975; Ball, 1983; Bal and Pike, 1984; King *et al.*, 1989; Thakur *et al.*, 1992; Thakur and Shetty, 1993) indicated the high degree of genetic divergence for virulence in *S. graminicola* populations that enable them to match resistant genes rapidly in the host genotypes.

Shorter latent periods were generally observed with the more aggressive isolate on highly susceptible host genotypes (Table 6). However, the isolates varied greatly for latent period (7.13-10.00 days) even on host genotype IP 18297, which recorded the lowest mean disease incidence (0.13%). Isolate Sg 140 produced disease incidence of 0.36 per cent on this genotype with a latent period 7.13 days which is relatively low. A host genotype, such as IP 18297, having fewer infected plants with shorter latent period could

contribute more towards disease spread than those having a greater number of infected plants with longer latent period. It was suggested that, the lower disease incidence alone can not be taken as a true measure of resistance in a highly variable pearl millet-downy mildew system (Thakur *et al.*, 1997). However, highly significant negative correlations between disease incidence and latent period provided a reasonable basis of understanding stability of resistance in the host genotypes.

In the present study, virulence index has been used to indicate the relative potential of individual isolates by combining the two independent pathogenicity parameters, disease incidence and latent period. It is quite variable among the isolates, across the host genotypes indicating the presence of different virulence genes in the pathogen populations to the corresponding resistance genes in the host differentials. Generally, all the isolates had highest virulence index values on 7042S, lowest on IP 18297 and P 3281-1, and variable on the remaining host genotypes (Table 9; Fig.8). Host genotypes on which the virulence index was lower across a number of isolates would probably be more stable than those with higher virulence index values. Of the 21 isolates evaluated, isolate Sg 139 had the highest virulence index (7.55) across the host genotypes indicating the highly virulent nature of the isolate. Isolate Sg 110, the least aggressive isolate had the lowest virulence index (2.04) suggesting that, such isolates being less fit could eventually be eliminated from the populations in the successive generations of the pathogen.

Except host genotypes P 1564 and IP 18292, all other compatible isolate-genotype combinations supported oospore production (Table 8). In addition, these two genotypes developed similar disease symptoms, which

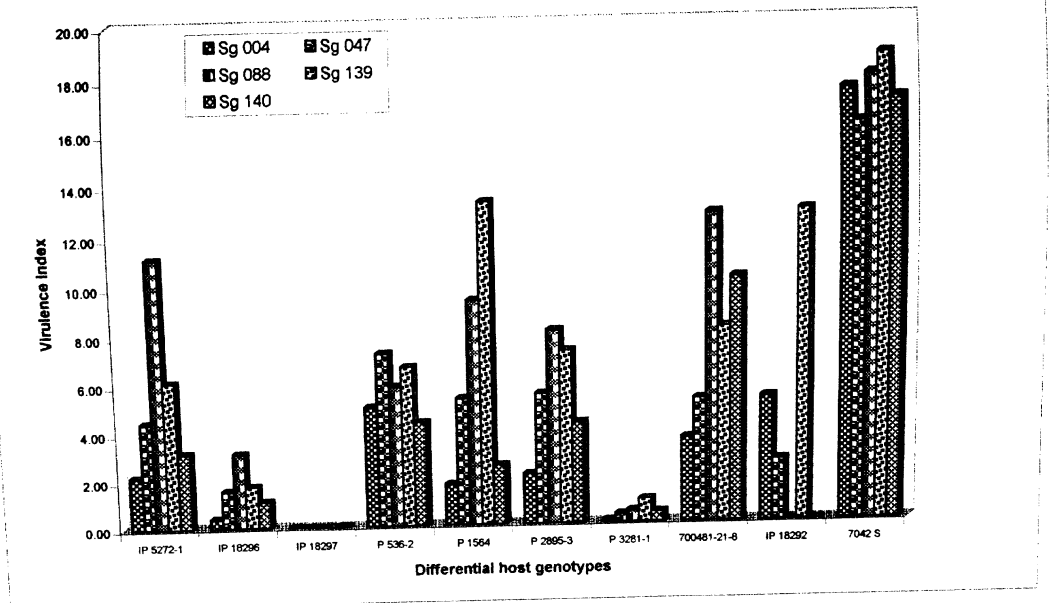

Fig. 8 : Virulence index of selected parental isolates on 10 differential host genotypes

include dark green foliage with severe stunting and no asexual sporulation on leaf lamina. The inability to support oospore production and expression of stunting reaction by these two genotypes indicate that they probably have a common resistance gene(s). The higher oospore production observed with some isolates (Sg 046, Sg 047, Sg 048, Sg 088, Sg 139, Sg 150 and Sg 153), irrespective of their virulence levels, could be attributed to the existence of differential mating types, Mat A and Mat B in equal proportions and it was proved in case of isolates *viz.*, Sg 048, Sg 139 and Sg 153 in which the frequency and distribution of mating types were demonstrated (Table 21). These findings are in conformity with the observations made by Michelmore and Ingram (1980) who obtained maximum oospore production when conidia of two mating types of *B. lactucae* were used in equal proportions. On the other hand, the host genotype, IP 18297 that showed resistant reaction (0.13% disease incidence) to all the isolates surprisingly supported relatively more oospore production. This observation indicates that, the oospore production potential may not be only pathogen-specific, but also host-specific in nature.

Based on disease incidence, 21 parental isolates were classified into seven distinct pathotype groups indicating significant variability among the populations of *S. graminicola*. No significant geographical distribution of isolates was found and isolates of the same state were assigned to different clusters. However, virulence analysis based on pathogenicity using differential host lines seems useful than molecular analysis in determining race structures of plant pathogens (Casela and Ferreira, 1995). As isolates from all pearl millet growing areas of the country were included in the present study, screening breeding material against the representative isolates of these pathotype groups

would provide stable resistance for a successful plant breeding programme in India.

Variation among single-zoospore isolates (SZIs)

The SZIs of *S. graminicola* derived from two parental isolates, Sg 139 (highly virulent) and Sg 110 (weakly virulent) differed significantly for disease incidence, latent period and virulence index on differential hosts. These results indicate great genetic variability potential within the populations of *S. graminicola*. Similar findings of intra-population variation in pathogenicity have also been reported by Caten and Jinks (1968) in *Phytophthora infestans*, Mathur *et al.* (1997) in *Colletotrichum sublineolum* and Thakur *et al.* (1998b) in *S. graminicola*. All the SZIs evaluated were highly aggressive on 7042S, either avirulent or weakly aggressive on IP 18297 and had variable reactions on the remaining host differentials. Among the SZIs of Sg 139, the isolate Sg 139-4 was highly aggressive and the isolate Sg 139-1 the least aggressive across the host genotypes (Table 9) whereas, among the SZIs of Sg 110 the isolate Sg 110-3 was highly aggressive and Sg 110-9 the least aggressive across the host genotypes (Table 13). Though significant variation was found among the SZIs of both the parental isolates, the degree of variation was more in case of Sg 139 than that of Sg 110. It is also evident from the results that certain SZIs showed high aggressiveness than their parental isolates on certain host genotypes. This indicates the evolution of progenies in a pathogen population with higher aggressiveness through sexual recombination. Similar findings have been reported in some populations of *Bremia lactucae* (Dixon and Wright, 1976; Lebeda, 1979; Gustafsson *et al.*, 1985).

The SZIs also varied greatly for latent period across the host genotypes similar to the parental isolates. This reflects the availability of an array of genes for resistance in host genotypes to the corresponding virulence genes in pathogen populations. The analysis of variance indicated significant differences for latent period in isolates, genotypes and their interactions, and this was attributed more to the isolates than to genotypes or isolate-genotype interactions. In contrast, Rao *et al.* (1998) found that the variation in latent period in isolates of *Colletotrichum graminicola* was attributed more to the host genotypes than to genotype or isolate- genotype interactions.

Though significant differences for virulence index were observed for most of the SZIs across the host genotypes, it is evident from the data that the differences for virulence index among the SZIs of Sg 139 were relatively more than those of Sg 110 (Tables 12 and 16; Figs. 9 and 10). This showed more diverse nature of the SZIs of Sg 139 than those of Sg 110, which could be due to the uniform distribution of mating types within the population of Sg 139 against erratic distribution of mating types in Sg 110 (Table 21). In a pathogen like *S. graminicola* distribution of mating types plays an important role in sexual reproduction which ultimately affect the evolution of new variants with matching virulence genes to the existing R-genes in the host cultivars (McDonald and Linde, 2002). These results also indicate the potential of development of more virulent isolates, which may have implications in epidemiology of disease and resistance stability in pearl millet. However, evidences exist for evolution of cultivar-specific virulences in populations of *S. graminicola* (Thakur *et al.*, 1992) and several promising cultivars such as HB1, HB3, BJ104 and MBH 110 have succumbed to downy mildew and have

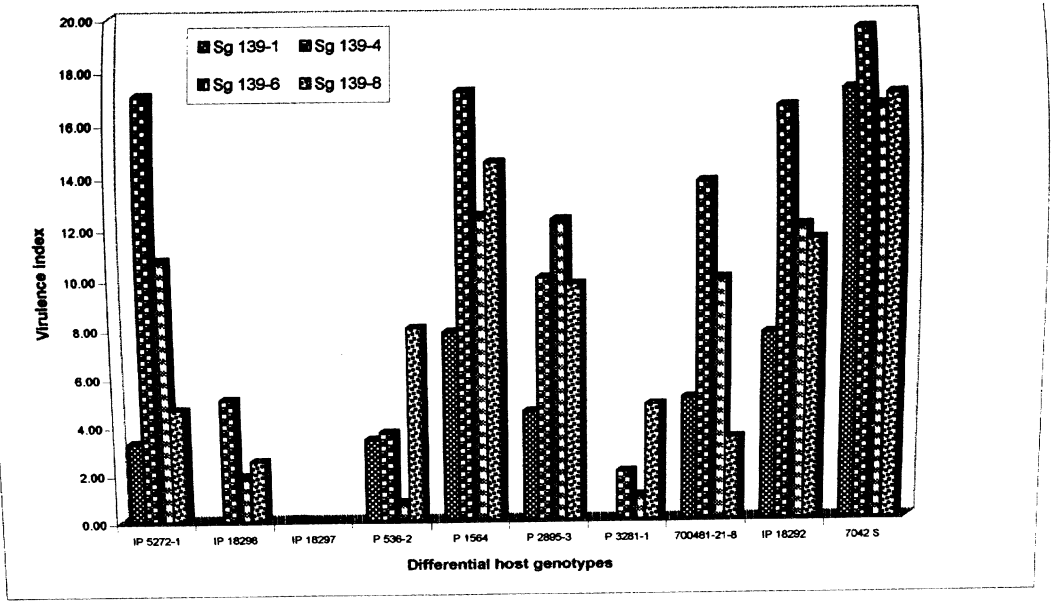

Fig. 9 : Virulence index of selected single-zoospore isolates of Sg 139 on 10 differential host genotypes

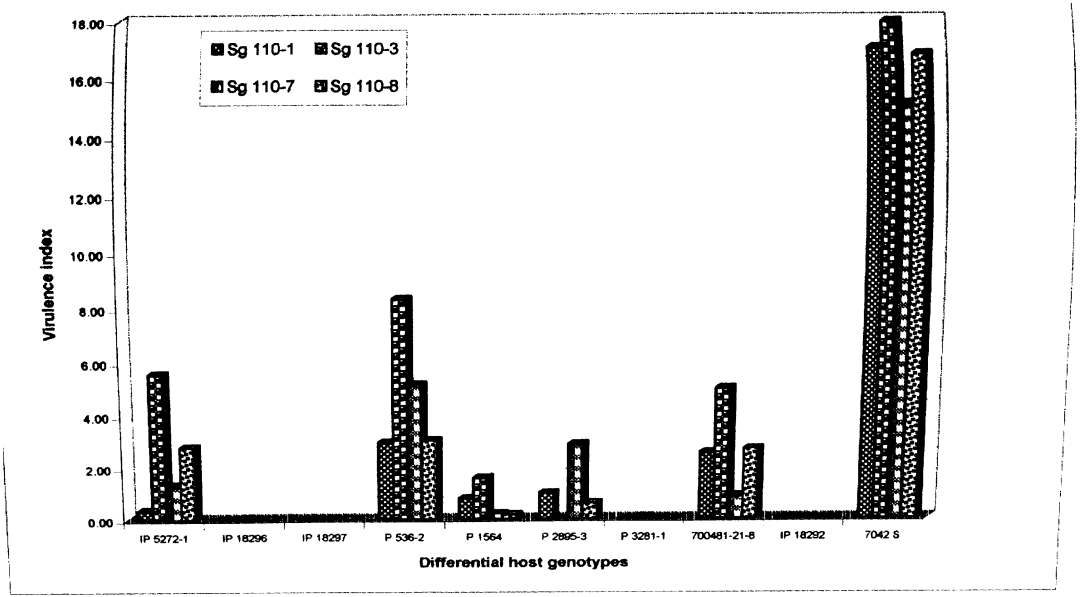

Fig. 10 : Virulence index of selected single-zoospore isolates of Sg 110 on 10 differential host genotypes

been withdrawn from cultivation (Singh *et al.*, 1997; Thakur, 1999). Evidences also exist for evolution of intra-population variation in pathogenicity between sister conidial lines from single lesion and monoconidial cultures in other pathogens, including *C. sublineolum* (Mathur *et al.*, 1997), *Fusarium* spp. (Sutton, 1980) and *Pyricularia oryzae* (Ou and Ayad, 1968).

Based on the disease incidence, the SZIs of Sg 139 were classified into five pathotype groups and those of Sg 110 into four pathotype groups with some variations within a group. Similar observations were made by Thakur *et al.* (1998b) who identified five pathotype groups while working with SZIs of *S. graminicola* derived from a parental isolate.

5.2 ASSESSMENT OF GENETIC DIVERSITY AMONG THE ISOLATES OF *S. graminicola*

Based on DNA polymorphism from AFLP analysis the 20 parental isolates of *S. graminicola* were classified into eight distinct groups. Fingerprinting pattern from AFLP analysis showed more diversity than virulence analysis based on pathogenicity using host differentials. The cluster composition also varied for virulence analysis and AFLP analysis. This is expected because gene(s) controlling a particular character is most likely to present in a small fraction across the genome, whereas the molecular banding pattern obtained from the total DNA reflects diversity within the entire genome (Andebrhan and Furtek, 1994). Similar observations were made by Chen *et al.* (1993) who found a high degree of molecular polymorphism among the isolates of *Puccinia striiformis* that had the same virulence phenotype and concluded that the molecular polymorphism observed was largely independent of virulence polymorphism. In the present study, the DNA polymorphism also

did not reflect the geographical distribution of isolates. Similar observations have been reported by Ouellet and Seifert (1993) in case of *Fusarium graminearum*, Casela *et al.* (1992 and 1995) in *Colletotrichum graminicola*, and Sivaramakrishnan *et al.* (2002a) in *Fusarium udum*, though in some cases importance of geographical regions were correlated (Guthrie *et al.*, 1992).

The inability of isolates Sg 004, Sg 025, Sg 026, Sg 139 and Sg 151 to form into clusters could be due to the presence or absence of few unique bands in these isolates (Plates 3-5). Separation of Sg 139 from other clusters in both, pathogenic and genetic analyses might be an indication of its highly virulent nature. The banding pattern obtained for this isolate can be used in future studies to develop Sequence Tagged Sites (STS) markers which enable the identification of virulent isolates of *S. graminicola* by using simple PCR technique which is rapid and also less expensive.

5.3 IDENTIFICATION OF MATING TYPES AMONG THE ISOLATES OF *S. graminicola*

Of the 70 single-zoospore isolates evaluated 62 were self-sterile and eight self-fertile (Table 18). These results indicate the predominant occurrence of self-sterility with the exception of few self-fertile isolates in populations of *S. graminicola*. The pattern of production of oospores by pairwise inoculations of 16 self-sterile isolates of *S. graminicola* suggested the existence of two distinct mating type groups, Mat A and Mat B. Crosses within each group were sterile, whereas those between them were fertile (Table 19). These observations confirm the findings of Michelmore *et al.* (1982) and illustrates the heterothallic nature of the fungus, *S. graminicola*. In addition, the results show that the frequency of the mating types, Mat A and Mat B in populations of

S. graminicola was approximately equal (Tables 20 and 21; Fig.11). In other heterothallic oomycetes also, only two sexual compatibility types have been identified in an approximate 1:1 ratio (Savage *et al.*, 1968; Brasier, 1969; Pratt and Green, 1973; Michelmore and Ingram, 1980). Since both the mating types were found approximately in equal proportion in India, it indicates that the sexual stage plays an important part in evolution of new genetic recombinants and thus development of new virulent pathotypes, and also helps in longterm survival of *S. graminicola* in the country.

The fungus is also known to have cross-compatibility between the isolates from geographically diverse locations in West Africa and India (Idris and Ball, 1984). This outbreeding capacity of *S. graminicola* is indicative of evolution for potential new races and high adaptability to different ecosystems (McDonald and Linde, 2002). Under these circumstances if oospores were inadvertently transported from one continent to the other, the pearl millet crop would be at risk. Pathogen isolates previously absent might proliferate rapidly in the absence of appropriate resistance factors in the host and may cause complete devastation of the crop.

Though the fungus was found primarily heterothallic in nature, the sparse oospore production by eight of SZIs when inoculated alone suggests the rare occurrence of self-fertility in populations of *S. graminicola*. Michelmore and Ingram (1982) demonstrated this self-fertility in *B. lactucae* as a form of secondary homothallism and not as a mixture of heterothallic isolates of opposite compatibility types. Such self-fertile isolates have been reported for other heterothallic members of the peronosporales : *Phytophthora* spp. (Mortimer *et al.*, 1978), *Pythium sylvaticum* (Pratt and green, 1973) and

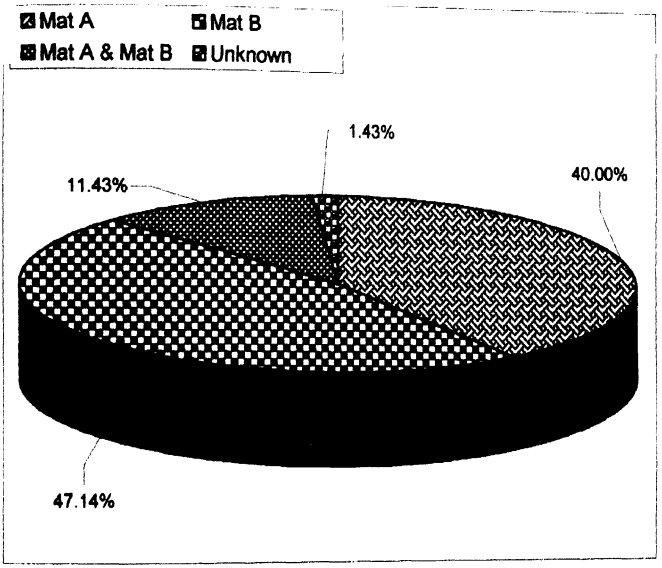

Fig. 11 : Composition, frequency and distribution of mating types among the isolates of *Sclerospora graminicola*

Peronospora parasitica (Sheriff and Lucas, 1989). However, the most detailed studies have been made with *P. drechsleri* (Mortimer *et al.*, 1978; Sansome, 1980) and *B. lactucae* (Michelmore and Ingram, 1982) where genetic and cytological experiments have indicated that the self-fertile isolates are trisomic for the determinants of compatibility type following numerical non-disjunction at meiosis. The self-fertility observed in the present investigation in some populations of *S. graminicola* may therefore be a similar form of secondary homothallism as described in *B. lactucae* (Michelmore and Ingram, 1982).

Among the 70 single-zoospore isolates analyzed for mating type behaviour, 28 (40.00%) were of mating type Mat A and 33 (47.14%) of Mat B, and eight (11.43%) apparently behaved as both Mat A and Mat B while one (1.43%) behaved unique of its kind (Tables 20 and 21; Fig.11). All isolates, except Sg 110-2, were capable of sexual reproduction when paired either with isolate PT2 or PT3. Only isolates which were capable of reproducing sexually when inoculated alone produced oospores with both, PT2 or PT3. Similar results were also obtained with *B. lactucae* (Michelmore and Ingram, 1982). However, further experimentation using large number of isolates of *S. graminicola* from even more diverse locations, especially form West Africa, is needed to determine whether the incapability of oospore production of Sg 110-2 with either of the mating type isolates is due to the existence of some other mating types in nature.

5.4 INHERITANCE OF VIRULENCE IN *S. graminicola*

Inheritance of virulence in *S. graminicola* was studied by hybridizing two heterothallic isolates which differed in their virulence on a differential host genotype, IP 18292. This is the first systematic and comprehensive report of such a study in *S. graminicola* and no published data exist on this aspect. The

results (Tables 22 and 23) indicated the segregation of virulence of *S. graminicola* to resistance present in IP 18292. Virulence to match this specific resistance in IP 18292 did not segregate in the F_1 and BC_1 generations, but in the BC_2 generation there was a 1:1 segregation, and in the F_2 generation a 3:1 ratio of avirulence to virulence was found. Specific virulence to resistance in IP 18292, therefore, appears to be determined by a single pair of alleles (*A/a*) at a single locus with avirulence being dominant to virulence. These results are in broad agreement with earlier studies which have shown that specific avirulence in specialized biotrophic pathogens is usually controlled by single dominant genes (Day, 1974; Ellingboe, 1981). Similar observations on dominance of avirulence over virulence have also been reported for the inheritance of specific virulence in *B. lactucae* (Norwood *et al.*, 1983; Michelmore *et al.*, 1984).

The segregation pattern also suggest that the virulence to resistance in IP 18292 segregated in clear Mendelian ratio, and the parents differed in respect of single gene pair. The monogenic ratio of 3 avirulent : 1 virulent, thus obtained indicates the dominant nature of genes for avirulence/ virulence in the pathogen, isolates Sg 139-4 and Sg 110-9 to resistance/susceptibility in the tester host genotype IP 18292. This is a clear and strong evidence in support of a true gene-for-gene relationship between *S. graminicola* and *P. glaucum*. These findings are in conformity with the earlier reports of gene-for-gene relationship in several host-parasite associations, such as that between *B. lactucae* and lettuce (Michelmore *et al.*, 1984; Crute, 1987; Michelmore *et al.*, 1988), *Phytophthora infestans* and potato (Al-Kherb *et al.*, 1995) and *Melampsora lini* and flax (Flor, 1965; Statler, 1990). In contrast to a major R-gene in IP 18292, several quantitative trait loci (QTL) for resistance against diverse pathotypes

of *S. graminicola* in different pearl millet genotypes have been identified (Jones *et al.*, 2002).

A gene-for-gene relationship between host cultivars and pathogen isolates has been proposed as the determinant of specificity in more than 30 host-pathogen associations (Crute, 1985). In its simplest form, the gene-for-gene theory proposed by Flor (1956) states that each locus conditioning specific host resistance or susceptibility is matched by a complementary locus controlling specific avirulence or virulence in the pathogen. In the interaction between flax and flax rust studied by Flor, an incompatible interaction phenotype occurred when any one host resistance allele was matched by the corresponding pathogen avirulence allele. Resistance and avirulence were nearly always dominant. These general observations apply to other host-parasite associations for which gene-for-gene relationships have been demonstrated.

The results (Table 23) also provide further genetical, rather than cytological, evidence (Tommerup *et al.*, 1974; Michelmore and Sansome, 1982) that *S. graminicola* is diploid in the vegetative phase rather than haploid or polyploid and the genetic evidence to suggest that the fungus is diploid in its vegetative phase comes form the lack of segregation for virulence in the F_1 generation. These findings are supported by the observations made by Michelmore *et al.* (1984) in case of *B. lactucae* and by Tommerup (1981) in several other Oomycetes also.

CONCLUSIONS AND FUTURE STRATEGIES

The diversity of pathogenicity and DNA polymorphism observed in the present investigation emphasizes the variability in *S. graminicola*. Because of its highly variable nature and rapid adaptation ability, several promising cultivars have succumbed to the disease during the past 20 years in India and the process continues. Therefore, a well-planned strategy to monitor virulence changes in the pathogen and resistance breakdown in host cultivars, and identification and incorporation of novel resistance genes, will help in reducing the chances of epidemics and losses from downy mildew in pearl millet.

For a successful plant breeding programme in the country, the breeding material should undergo thorough screening using all available potential pathotypes of *S. graminicola*. To identify such pathotypes at right time, in a right way, it is essential to develop a series of good near-isogenic lines containing different downy mildew resistance genes (R genes) which seems to be a prerequisite for identification of new virulences in *S. graminicola* populations.

The mating type behaviour and rare occurrence of self-fertility in *S. graminicola* populations observed in the present investigation indicate the high potential of the pathogen to provide new genetic recombinants to challenge the resistant genes of the host. Such studies, should be conducted involving larger number of isolates sampled from geographically diverse areas and genetically diverse host cultivars to understand the changes in the mating type behaviour of the pathogen.

The gene-for-gene hypothesis observed in the present study appears to be an adequate genetic description of most differential interactions in pearl millet - downy mildew pathosystem. However, there may be complexities such as inhibitor and modifier genes superimposed on the one-to-one complementarity of host and pathogen genes and such deviations can be revealed by making more number of crosses between all possible virulence phenotypes.

Summary

CHAPTER-VI

SUMMARY

In the present investigation, studies pertaining to pathogenic and genetic diversity, mating types and inheritance of virulence in *S. graminicola* the causal agent of downy mildew of pearl millet were carried out. All the experiments were conducted at ICRISAT, Patancheru, Andhra Pradesh, India. The results obtained are summarized below.

A total of 21 isolates representing all important pearl millet growing areas of India were selected from the collections maintained at ICRISAT. The isolates were established on universally susceptible host genotype 7042S, in the form of asexual inocula from the oosporic inocula and were denoted as parental isolates.

Considerable variation was found among the isolates for disease incidence and latent period during the establishment of parental isolates. Of the 21 parental isolates, the isolate Sg 153 recorded highest disease incidence (76.15%) with shortest latent period (6.00 days), while the isolate Sg 004 (2.61%) and Sg 021 (1.97%) recorded lowest disease incidence with longest latent period (30.00 days). Isolates collected during 1997 recorded significantly high disease incidence than those collected in the previous years.

Twenty one parental isolates were evaluated for pathogenecity on a set of ten host differentials consisting *viz.*, IP 5272-1, IP 18296, P 536-2, P 1564, P 2895-3, P 3281-1, 700481-21-8, IP 18292 and 7042S. Isolates varied greatly for virulence, disease incidence, disease reaction, latent period, virulence index and oospore production potential. Among the parental isolates, the isolate Sg 139 was highly virulent with highest mean virulence index (7.55) and the isolate Sg 110 weakly

virulent with lowest mean virulence index (2.04). However, the oospore production rating was maximum in case of isolate Sg 153 (3.25) and the minimum for isolate Sg 115 (2.02).

Based on disease incidence, the parental isolates were classified into seven pathotype groups. A representative isolate from each group (Sg 048, Sg 149, Sg 021, Sg 110, Sg 153, Sg 139 and Sg 152) were selected. From each representative isolate, ten single-zoospore isolates (SZIs) were established for conducting further studies.

The SZIs derived from the highly virulent parental isolate, Sg 139 and the weakly virulent parental isolate, Sg 110 were evaluated for pathogenicity on host differentials to detect variability within the isolates of *S. graminicola*. All the SZIs varied significantly for virulence, disease incidence, disease reaction, latent period and virulence index. Among the SZIs of Sg 139, the isolate Sg 139-4 was found highly virulent (virulence index 10.47) and the least virulent was the isolate Sg 139-1 (virulence index 4.91), whereas Sg 110-3 was the highly virulent (virulence index 3.85) and Sg 110-9 was the least virulent (virulence index 1.57) among the SZIs of Sg 110. Based on the disease incidence, SZIs of Sg 139 were classified into five groups and those of Sg 110 into four groups.

The parental isolates, when subjected to AFLP analysis using three primer combinations (E-TG/M-CAT, E-TT/M-TAG and E-TG/M-CTA) showed a high degree of polymorphism at DNA level. The fingerprint pattern of AFLP analysis showed more diversity than virulence analysis. Based on similarity index, the isolates were clustered into eight groups. The cluster composition of AFLP analysis did not match with that of the virulence analysis, and these two were found independent. The classification of isolates by either of the analysis also did not show any lineage with the geographical distribution of the isolates.

Of the 70 SZIs tested for fertility, 62 were found self-sterile and 8 were self-fertile. From the 62 self-sterile isolates, 10 SZIs of Sg 139 and six SZIs of Sg 110 were used to detect mating types by inoculating them singly and in all possible paired combinations on the susceptible host genotype 7042S. The results indicated the existence of two mating types designated, Mat A and Mat B.

All SZIs were tested for their oospsore production potential by pairing with the two standard mating type isolates PT2 (Mat A) and PT 3 (Mat B) to determine the composition, frequency, and distribution of mating types among the isolates. Of the 70 SZIs tested, 28 (40.00%) were of Mat A, 33 (47.14%) of Mat B, eight (11.43%) of both Mat A and Mat B, and one (1.43%) behaved unique of its kind. The frequency of both the mating types among the isolates of *S. graminicola* found approximately in equal proportions. However, their distribution within the isolates of Sg 149, Sg 110 and Sg 152 was not uniform.

To determine the inheritance of virulence in *S. graminicola* a cross was made between the highly virulent SZI, Sg 139-4 (Mat A) and the weakly virulent SZI (avirulent on IP 18292), Sg 110-9 (Mat B) which differed in their virulence on host differential IP 18292. F_1, F_2 and backcross progenies were evaluated for their virulence phenotype on the tester host genotype. Avirulence was found dominant over virulence. Further, monogenic ratio (3 avirulent : 1 virulent) observed in F_2 generation indicated the role of single gene pair in governing the virulence in isolates Sg 139-4 and Sg 110-9 and resistance in IP 18292. The segregation pattern obtained also suggested a true gene-for-gene relationship between *S. graminicola* and *P. glaucum*.

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* : Original not seen

Appendices

APPENDIX I

Analysis of variance for per cent disease incidence of 21 parental isolates of *Sclerospora graminicola* on ten differential host genotypes

Source of variation	df	Mean sum of squares	F-value
Isolates (I)	20	1997.62	1698.27**
Host genotypes (H)	9	30494.49	25924.85**
I X H	180	451.54	383.87**
Residual	420	1.18	

** Significant at (P<0.01)

APPENDIX II

Analysis of variance for latent period of 21 parental isolates of *Sclerospora graminicola* on ten differential host genotypes

Source of variation	df	Mean sum of squares	F-value
Isolates (I)	20	28.37	600.35**
Host genotypes (H)	9	231.18	4892.67**
I X H	180	22.48	475.69**
Residual	420	0.05	

** Significant at (P<0.01)

APPENDIX III

Analysis of variance for virulence index of 21 parental isolates of *Sclerospora graminicola* on ten differential host genotypes

Source of variation	df	Mean sum of squares	F-value
Isolates (I)	20	70.46	833.16**
Host genotypes (H)	9	1364.30	16132.19**
I X H	180	15.07	178.20**
Residual	420	0.08	

** Significant at (P<0.01)

APPENDIX IV

Analysis of variance for oospore production ratings of 21 parental isolates of *Sclerospora graminicola* on ten differential host genotypes

Source of variation	df	Mean sum of squares	F-value
Isolates (I)	20	6.05	1150.16**
Host genotypes (H)	9	83.53	15893.09**
I X H	180	2.05	390.95**
Residual	420	0.005	

** Significant at (P<0.01)

APPENDIX V

Analysis of variance for per cent disease incidence of ten single-zoospore isolates of Sg 139 on ten differential host genotypes

Source of variation	Df	Mean sum of squares	F-value
Isolates (I)	9	1517.74	3809.97**
Host genotypes (H)	9	20324.99	51021.74**
I X H	81	308.40	774.18**
Residual	200	0.40	

** Significant at (P<0.01)

APPENDIX VI

Analysis of variance for latent period of ten single-zoospore isolates of Sg 139 on ten differential host genotypes

Source of variation	df	Mean sum of squares	F-value
Isolates (I)	9	18.48	1035.07**
Host genotypes (H)	9	90.54	5070.00**
I X H	81	14.09	788.87**
Residual	200	0.02	

** Significant at (P<0.01)

APPENDIX VII

Analysis of variance for virulence index of ten single-zoospore isolates of Sg 139 on ten differential host genotypes

Source of variation	df	Mean sum of squares	F-value
Isolates (I)	9	82.88	1606.34**
Host genotypes (H)	9	957.89	18565.02**
I X H	81	16.95	328.59**
Residual	200	0.05	

** Significant at (P<0.01)

APPENDIX VIII

Analysis of variance for per cent disease incidence of ten single-zoospore isolates of Sg 110 on ten differential host genotypes

Source of variation	df	Mean sum of squares	F-value
Isolates (I)	9	700.40	17076.35**
Host genotypes (H)	9	16071.15	391830.00**
I X H	81	165.09	4024.97**
Residual	200	0.04	

' Significant at (P<0.01)

APPENDIX IX

Analysis of variance for latent period of ten single-zoospore isolates of Sg 110 on ten differential host genotypes

Source of variation	df	Mean sum of squares	F-value
Isolates (I)	9	36.91	1184.42**
Host genotypes (H)	9	370.56	11892.46**
I X H	81	24.15	775.08**
Residual	200	0.03	

** Significant at (P<0.01)

APPENDIX X

Analysis of variance for virulence index of ten single-zoospore isolates of Sg 110 on ten differential host genotypes

Source of variation	df	Mean sum of squares	F-value
Isolates (I)	9	14.15	226.21**
Host genotypes (H)	9	737.59	11793.64**
I X H	81	3.32	53.15**
Residual	200	0.06	

** Significant at (P<0.01)