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## Inheritance of Avirulence in *Sclerospora graminicola*, the Pearl Millet Downy Mildew Pathogen

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**Abstract:** The present research studied the inheritance of avirulence by hybridizing the isolates Sg 139-4 (Mat A) a highly virulent and Sg 110-9 (Mat B) an avirulent on a pearl millet genotype IP 18292 containing a downy mildew resistance gene *Rsg1*. Pot-grown seedlings of IP 18292 were inoculated with sporangial inocula generated from sexual spores (oospore) of the parental isolates and their F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> and BC<sub>2</sub> progenies and incubated at 25±2°C and >90% relative humidity in a greenhouse. Downy mildew incidence was recorded two weeks after inoculation. The results indicated that avirulence was dominant over virulence and a single gene pair (AA/Aa) controlled avirulence in isolates Sg 110-9, to a corresponding resistance gene *Rsg1* in IP 18292. The pattern of segregation of virulence: avirulence suggested the presence of a gene-for-gene interaction between *S. graminicola* and *P. glaucum*.

**Key words:** Pearl millet, downy mildew, *Sclerospora graminicola*, avirulence, inheritance

### INTRODUCTION

Downy mildew of pearl millet [*Pennisetum glaucum* (L.) R. Br.], caused by *Sclerospora graminicola* (Sacc.) Schroet is a serious disease, particularly on single-cross F<sub>1</sub> hybrids, which are cultivated on about 60% of the total 10 million ha in India<sup>[1]</sup>.

During the 1970s and the 1980s several downy mildew epidemics occurred resulting in withdrawal of several hybrids<sup>[2,3]</sup>. Currently, over 50 different hybrid cultivars are grown in India and some of these have recorded downy mildew incidence up to 100% during the surveys in states of Maharashtra, Rajasthan and Gujarat<sup>[4-6]</sup>, accounting for a considerable yield loss.

*S. graminicola*, is an oomycetous obligate biotroph and reproduces both by sexual and asexual means. It is a heterothallic fungus and existence of mating types has been demonstrated<sup>[7-9]</sup>. High susceptibility of otherwise resistant hybrids within few years of cultivation led to the understanding of host-directed virulence selection in *S. graminicola* population<sup>[10]</sup>. Systematic survey of pearl millet hybrids in farmers' fields for downy mildew incidence, collection of isolates from susceptible hybrids and their pathogenic characterization using host differentials and the multilocation evaluation of pearl millet downy mildew virulence nursery have led to the

identification of at least six different populations (pathotypes) of *S. graminicola* existing in different parts of India<sup>[11,12]</sup>.

Resistance to downy mildew in pearl millet is governed by single dominant gene<sup>[13]</sup>, recessive gene<sup>[14]</sup> and multiple genes with epistatic effects<sup>[15]</sup>. A number of Quantitative Trait Loci (QTL) offering resistance to individual or multiple pathotypes have been detected in some of the resistance sources used in developing mapping populations<sup>[16,17]</sup>.

Studies on inheritance of avirulence are as important as those on inheritance of resistance to understand the host-pathogen interaction at the genetic level. Following the classical studies by Flor<sup>[18]</sup> and establishment of the gene-for-gene hypothesis for the inheritance of pathogenicity in the flax rust fungus, *Melampsora lini* several studies have been reported. Person and Sidhu<sup>[19]</sup> reviewed the subject on the genetics of pathogenicity and generalized that the virulence/avirulence was usually under Mendelian control in *Ustilago hordei* (Pers.), the barley smut pathogen. In maize, Lim *et al.*<sup>[20]</sup> reported monogenic inheritance of virulence for *Helminthosporium turcicum*. Studies on inheritance of virulence in the lettuce downy mildew pathogen *Bremia lactucae* demonstrated that the virulence to match resistance factors R<sub>1</sub>, R<sub>2</sub>, R<sub>4</sub> and R<sub>11</sub> each segregated as single loci

with avirulence being dominant to virulence and the inheritance of virulence to  $R_6$  was complex<sup>[21,22]</sup>. These studies confirmed that the virulence in *B. lactucae* to match the specific resistance genes located in lettuce cultivars was 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<sup>[21]</sup> and linkage was also suggested between loci controlling virulence to  $R_5$ ,  $R_8$  and  $R_{10}$ <sup>[22]</sup>. Such studies in case of *S. graminicola* are lacking, primarily because of the unavailability of well-defined resistance genes in its host, pearl millet. In this study, we investigated the inheritance of avirulence using two distinct single-zoospore isolates, Sg 139-4 and Sg 110-9 of *S. graminicola* that were clearly defined as virulent and avirulent, respectively on a pearl millet inbred line IP 18292 that was shown to contain a single dominant gene (*Sgr1*) conferring complete resistance to a specific downy mildew pathotype<sup>[13]</sup>.

## MATERIALS AND METHODS

**Selection of parental isolates of *S. graminicola* and tester host genotype:** The highly virulent Single-zoospore Isolate (SZI), Sg 139-4 (Mat A) and avirulent isolate SZI Sg 110-9 (Mat B) on a pearl millet genotype IP 18292, a pure inbred line, were selected as parental isolates for the study. The SZIs were maintained on seedlings of a uniformly highly susceptible pearl millet genotype 7042S through asexual generation in isolation chambers in a greenhouse<sup>[23]</sup>.

**Hybridization:** The pot-grown seedlings of 7042S at the coleoptiles to first-leaf stage were spray-inoculated with a sporangial suspension ( $5 \times 10^7$  sporangia  $\text{mL}^{-1}$ ) containing inocula in equal (1:1) proportion of Sg 139-4 and Sg 110-9. Fifty pots of 15 cm dia with 10 seedlings per pot were maintained and infected seedlings were grown for a month to obtain sufficient  $F_1$  oospores.

**Establishment and evaluation of  $F_1$  progenies:** Sixty days after inoculation, necrotic leaves from the infected plants were collected, dried and ground into powder. The leaf powder thus obtained contained  $F_1$ -oospores. A low frequency of seedling infection from  $F_1$ -oospores was obtained by adding 0.25 g of leaf powder containing oospores to the autoclaved soil contained in 15 cm dia pots that were sown with 7042S, 50 seeds  $\text{pot}^{-1}$ . As infected seedlings occurred infrequently and rarely, each infected seedling was assumed to have resulted from infection by single oospore. Sporangial inoculum from each seedling was maintained separately on 7042S as an

individual  $F_1$  progeny isolate in a polyacrylic isolation chamber (60×60×90cm) in a greenhouse at  $25 \pm 2^\circ\text{C}$ . A total of 33  $F_1$  progeny isolates were established and each progeny isolate was tested for its virulence phenotype (pathogenicity reaction) by inoculating 100-120 pot-grown seedlings of the tester host genotype, IP 18292 and 100-120 pot-grown seedlings of the maintainer host genotype 7042S. Each time a group of  $F_1$  progeny isolates (usually 10) along with their parental isolates, Sg 139-4 and Sg 110-9 were tested for comparison, convenience of handling and data recording. Because the downy mildew infection is systemic, data were recorded for the percentage of seedlings infected two weeks after inoculation.

**Establishment and evaluation of  $F_2$  progenies:** The  $F_1$  progeny isolates were allowed to produce oospores on 7042S until 60 days after inoculation. Since all the  $F_1$  progeny isolates exhibited avirulent reaction on the tester host genotype IP 18292, the necrotic leaves from 7042S collected for all the  $F_1$  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 on 7042S as described above. A total of 230 single-oospore  $F_2$  progeny isolates were established and maintained, separately on seedlings of 7042S in isolation chambers in the greenhouses.

Each  $F_2$  progeny isolate was tested for its virulence phenotype in terms of disease incidence on 100-120 seedlings (2 pots) of the tester host genotype IP 18292 and 100-120 seedlings of the maintainer genotype 7042S. Based on disease incidence (DI) on IP 18292, the  $F_2$  progeny isolates were classified into either virulent (>10% DI) or avirulent (<10% DI) group. The data obtained were used to assess the segregation pattern. Each time the virulence phenotypes of a group of  $F_2$  progeny isolates (usually 10) with the two parental isolates, Sg 139-4 and Sg 110-9, were tested for the convenience of handling and data recording.

**Backcrosses:** Of the 33  $F_1$  progeny isolates, two isolates with different compatible mating types were identified by hybridizing with the reference mating types<sup>[9]</sup> and crossed with the original parents of the complementary mating type by inoculating them onto seedlings of 7042S. Forty-six progeny isolates from backcross 1 ( $F_1 \times$  Sg 110-9) and 62 progeny isolates from backcross 2 ( $F_1 \times$  Sg 139-4) that produced oospores were recovered. From these asexual progenies (sporangial inocula) were raised on 7042S and evaluated for virulence phenotype on the tester host genotype, IP 18292.

A schematic representation of various steps involved in the study is presented in Fig. 1 and the number of seedlings inoculated in Table 1.

**Statistical analysis:** The goodness-of-fit of the segregation ratio of virulence phenotypes and avirulence phenotypes to the theoretical ratio was tested using Chi-square analysis<sup>[24]</sup>.

**RESULTS AND DISCUSSION**

**Evaluation for virulence:** The parental and the F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> and BC<sub>2</sub> progeny isolates were evaluated for virulence phenotype on the tester host genotype IP 18292 and the maintainer host genotype 7042S. The two parental isolates, Sg 139-4 and Sg 110-9 caused 97.4% and 0.0% incidence, respectively on IP 18292 and 95.6 and 94.7%, respectively on 7042S (Table 1). These remained true to their virulent and avirulent types. All the 33 F<sub>1</sub> progeny isolates caused no disease on the tester genotype IP 18292 and thus remained avirulent, while these caused 96.1% incidence on 7042S. The 230 F<sub>2</sub> progeny isolates caused mean disease incidence of 20.5% on IP 18292 and 92.5% on 7042S (Table 1). Based on the frequency distribution for disease causing potential on IP 18292, the 230 isolates were classified into 177 avirulent and 53 virulent with incidence ranging from 51.0 to 100.0% (Table 2). This segregation ratio of 177 avirulent: 53 virulent did not differ significantly from the Mendelian

Table 1: Details of seedlings inoculated by *Sclerospora graminicola* isolates and the percentage seedlings infected

Parent/Filial generation	No of progeny	Tester/ main-tainer host genotype	No. of seedlings inoculated	Percentage of seedlings infected
Sg 139-4 (P <sub>1</sub> )	1	IP 18292 (124 pots)	112	97.4
		7042S (2 pots)	114	95.6
Sg 110-9 (P <sub>1</sub> )	1	IP 18292	108	0.0
		7042S (2 pots)	112	94.7
F <sub>1</sub> (P <sub>1</sub> × P <sub>2</sub> )	33	IP 18292 (66 pots)	3730	0.0
		7042S (66 pots)	3654	96.1
F <sub>2</sub>	230	IP 18292 (460 pots)	25010	20.5
		7042S (460 pots)	25870	92.5
BC <sub>1</sub> (F <sub>1</sub> × P <sub>1</sub> )	46	IP 18292 (92 pots)	4822	0.0
		7042S (92 pots)	4945	96.0
BC <sub>2</sub> (F <sub>1</sub> × P <sub>2</sub> )	62	IP 18292 (124 pots)	6631	49.1
		7042S (124 pots)	6586	95.5

\*Plastic pots 15 cm dia with 50-60 seedlings per pot, thinned to 10 seedlings per pot after recording disease reaction and maintained until 60 days after sowing to obtain sexual (oospores) for F<sub>1</sub> and F<sub>2</sub> generations

Table 2: Disease incidence range and frequency of virulent/avirulent phenotypes in F<sub>2</sub> generation

No. of F <sub>2</sub> progenies	Disease incidence (%) class	Av/v phenotype
177	0-10	Av
1	51-60	v
1	61-70	v
4	71-80	v
13	81-90	v
34	91-100	v

Av = Avirulent with ≤10% incidence; v = virulent with >10% incidence

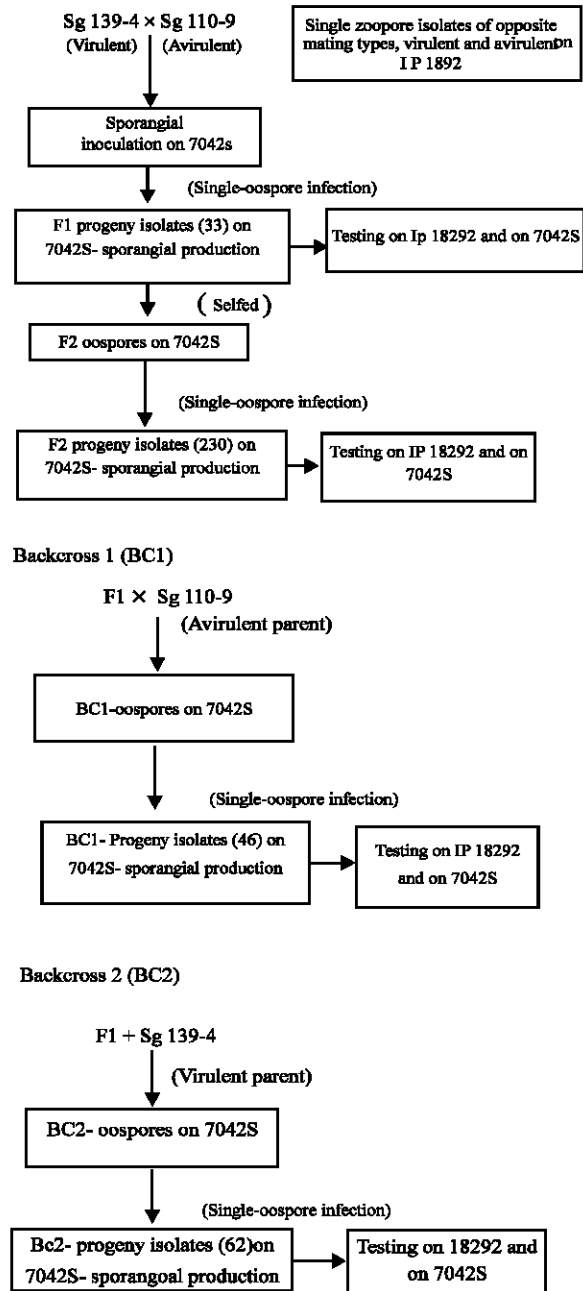


Fig. 1: Schematic representation of inheritance of a virulence study in *Sclerospora graminicola*

single gene inheritance ratio of 3:1 ( $\chi^2$  3:1 = 0.46, P>0.01) (Table 3). The 46 BC<sub>1</sub> progeny isolates (F<sub>1</sub> × P<sub>1</sub>) did not cause disease on IP 18292 and remained truly avirulent, while they caused 96% incidence on 7042S (Table 1). However, the 62 BC<sub>2</sub> (F<sub>1</sub> × P<sub>2</sub>) progenies caused 49.1% disease on IP 18292 (Table 1) and they segregated into 28 avirulent: 34 virulent which showed a good fit to a 1:1

Table 3: Observed and expected segregation of virulence phenotypes of F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> and BC<sub>2</sub> progeny isolates of *Sclerospora graminicola* together with assumed genotypes

Isolates	Progeny	Observed segregation	Expected segregation	Assumed genotypes		$\chi^2$
		Avirulent: Virulent	Avirulent: Virulent	Avirulent	Virulent	
Sg 110-9 (Avirulent parent)	1	-	-	AA	-	-
Sg 139-4 (Virulent parent)	1	-	-	-	aa	-
F <sub>1</sub> progeny	33	33:0	1:0	Aa	-	-
F <sub>2</sub> progeny	230	177:53	3:1	AA and Aa	aa	0.46
BC <sub>1</sub> progeny	46	46:0	1:0	AA and Aa	-	-
BC <sub>2</sub> progeny	62	28:34	1:1	Aa	aa	0.58

A: Dominant avirulent allele, a: Recessive virulent allele,  $\chi^2$ : Values are not significant (p>0.01)

segregation ratio ( $\chi^2$  1:1 = 0.58, p>0.01) (Table 3). Thus the monogenic segregation pattern (3:1) of F<sub>2</sub> generation was supported by the segregation ratios obtained in BC<sub>1</sub> (no segregation) and BC<sub>2</sub> (1:1 segregation) generations.

**Inheritance of avirulence:** It is evident from the results 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 being dominant over virulence (Table 3). The segregation pattern also suggests that, the parents are homozygous and the F<sub>1</sub>s are heterozygous at this locus. The avirulent parent Sg 110-9 and the virulent parent Sg 139-4 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 probable genotypes of parents, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub> and BC<sub>2</sub> generation isolates are given in Table 3. This is the first study on inheritance of avirulence in the *S. graminicola* and *P. glaucum* system. These results are in broad agreement with earlier studies on different host-pathogen systems, which have shown that specific avirulence in specialized biotrophic pathogens is usually controlled by single dominant genes<sup>[25,26]</sup>. Similar observations on dominance of avirulence over virulence have also been reported for the inheritance of specific virulence in *B. lactucae*, the downy mildew pathogen of lettuce<sup>[21,22,27,28]</sup>.

The results provide clear 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 other host-parasite associations, such as that between *B. lactucae* and lettuce<sup>[22]</sup>, *Phytophthora infestans* and potato<sup>[29]</sup> and *M. lini* and flax<sup>[30,31]</sup>. In contrast to a major R-gene (*Sgr1*) in IP 18292 offering complete resistance to a specific pathotype<sup>[13]</sup>, several QTL for resistance against diverse pathotypes of *S. graminicola* in different pearl millet genotypes have been identified<sup>[1,32]</sup>. Thus

virulence/resistance in *S. graminicola*-pearl millet system could be both qualitative and quantitative depending on the pathotype isolate and the host genotype combinations.

The lack of segregation for virulence in the F<sub>1</sub> generation provides further evidence that *S. graminicola* is diploid in the vegetative phase rather than haploid or polyploid. These findings are similar to those reported by Michelmore *et al.*<sup>[22]</sup> in case of *B. lactucae* and by Tommerup<sup>[33]</sup> in several other oomycetes. Further studies using other pathotype isolates of *S. graminicola* would be needed to better understand the genetics and virulence mechanisms in the pearl millet-*S. graminicola* system.

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