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Variation in virulence and aggressiveness among pathotypes of *Sclerospora graminicola* on pearl millet

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ABSTRACT : Five pathotypes of *Sclerospora graminicola,* specific to pearl millet (*Pennisetum glaucum*) genotypes, were evaluated for virulence (a qualitative measure of the relative capacity of an isolate to infect a host genotype), aggressiveness (a quantitative measure of the infection causing potential of an isolate, calculated as disease incidence), and virulence index (a quantitative measure of virulence, measured as disease incidence × latent period^{-1}) on a set of pearl millet genotypes in a glasshouse. The pathotypes were selected from a field population of the pathogen through a number of successive asexual generations on specific host genotypes. These were named as pathotype, Path 1 (host NHB 3), Path 2 (BJ 104), Path 3 (MBH 110), Path 4 (852B) and Path 5 (a field population from a mixture of NHB 3 and 7042S). The pathotypes differed significantly for virulence, aggressiveness and virulence index on the host genotypes. Highly significant ($P<0.001$) pathotype × host genotype interaction effects for virulence, aggressiveness, and virulence index indicated the existence of host-pathogen specificity in the *P. glaucum-S. graminicola* system. Differential interactions for virulence were evident on eight of the 14 host genotypes. All five pathotypes were moderately virulent (3.75-7.03 virulence index) and less aggressive (28-46% incidence) on ICMP 85410 indicating the presence of non-pathotype-specific resistance which could be more stable. The other pearl millet genotypes (IP 18292, IP 18293, 7042R, P 7-4, and P 310-17), to which the pathotypes were either avirulent or less virulent, can serve as sources of stable resistance.

Keywords : *Sclerospora graminicola,* pearl millet, virulence, aggressiveness

Sclerospora graminicola [Sacc.} Schroet.] is the causal agent of downy mildew, the most destructive and widespread disease of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. The disease causes significant losses (10-70%) in grain yield (Singh *et al.,* 1993), and is considered as the major constraint of high yield in single-cross pearl millet hybrids (Andrews *et al.,* 1985; Rachie and Majmudar, 1980; Williams, 1984). In India, downy mildew epidemics have been frequent in the commercial hybrids resulting in heavy economic losses and subsequent withdrawal of hybrids from cultivation (AICPMIP, 1977-92). In contrast, heterogeneous open-pollinated varieties have been found to be more durable over time in maintaining disease resistance (AICPMIP, 1977-92; Singh *et al.,* 1993). Currently, efforts to improve resistance and yield in pearl millet cultivars have been based on utilization of resistance in breeding hybrids and open-pollinated varieties (Andrews *et al.,* 1985). *S. graminicola* is an obligate biotroph and heterothallic (Michelmore *et al.,* 1982), and therefore genetically highly variable. Pathogenic variation among isolates of *S. graminicola* from different parts of Africa and India has been reported (Ball,

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1983; Ball and Pike, 1984; King *et al.*, 1989; Shetty and Ahmed 1981; Singh and Singh, 1987; Thakur and Rao, 1993; Thakur and Shetty, 1993).

The hypothesis of host genotype-directed virulence selection was tested and two pathotypes with specific virulence to pearl millet genotypes MBH 110 and 852B were selected from an initially less virulent field population of *S. graminicola* (Thakur *et al.*, 1992).

The objectives of the present study were to determine variation for virulence and aggressiveness among five pathotypes of *S. graminicola* on a set of pearl millet genotypes, and identify host genotypes with likely stable resistance.

MATERIALS AND METHODS

Inoculum and inoculation

Downy mildew infected leaves of pearl millet were harvested, and wiped clean with a wet cotton swab in running tap water to remove old spores and other contaminants. These leaves were cut into 4-5 cm pieces, placed in moist chamber in plastic trays lined with blotting paper and incubated at 20°C for 6 h in the dark for sporulation. Sporangia were harvested from the leaf pieces in chilled (5°C) sterilized distilled water with the help of a camel hair brush. The resulting sporangial suspension was adjusted to about 1×10^5 sporangia ml^{-1} for inoculation. Seedlings at the 2-leaf stage (4-5 days after sowing) were spray-inoculated until run-off with the sporangial suspension and then allowed to dry for 15 min. The inoculated seedlings were incubated in the dark at 20°C under high relative humidity (>95%) for 24 h and later transferred to the greenhouse at 25±2°C. All 14 pearl millet genotypes (Table 1) were inoculated with each of the five pathotype isolates of *S. graminicola.*

Selection of host genotype-specific pathotypes

Downy mildew infected leaves, containing abundant oospores, were collected from highly susceptible pearl millet genotypes NHB 3 and 7042S in the ICRISAT Asia Center (IAC) downy mildew field nursery. The leaves were dried in shade and ground to obtain oospores in the leaf powder. A potting mixture of autoclaved soil, sand and farm yard manure (3:2:2 by volume) contained in plastic pots (15-cm diameter) was infested by mixing 2 g leaf powder in the top 2-cm layer of the mix in each pot. Between 25 and 30 seeds were sown in each pot and watered regularly. For each of the four pearl millet genotypes (hybrids: NHB 3, BJ 104, MBH 110; and inbred: 852B) about 40 plants were maintained in two pots in separate fibre-glass isolation chambers in a greenhouse at 25±2°C to eliminate any cross contamination. Infected seedlings, about 2 weeks after inoculation, were marked in each pot and healthy seedlings were removed. Sporangia from these infected seedlings were used for subsequent inoculation of seedlings of the respective host genotypes. Repeated inoculation of seedlings, once a month for each host genotype with sporangia from the respective host continued until the frequency of infected plants stabilized. This process took only two generations for pathotype, (Path 1 (on NHB 3), five each for Path 2 (on BJ 104) and Path 4 (on 852B), and 25 for Path 3 (on MBH 110) (Fig. 1). At the time of this study, 30 generations of different pathotypes were grown on the respective selection host genotypes. These pathotypes produced only sporangia,

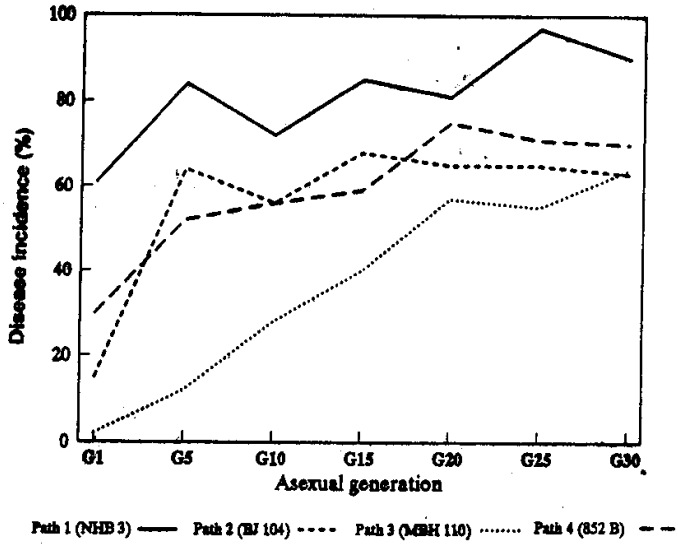

Fig. 1. Infection frquency (%) induced by pathotypes of *Sclerospora graminicola* on their selection host (pearl millet genotypes) at different asexual generations in a greenhouse experiment.

and no oospores were detected in the infected host tissues after two to four initial generations. We assumed that each pathotype had one mating type and the population was nearly homogeneous. The pathotypes were continued to be maintained on their respective selection host genotypes through successive asexual generations in isolation chambers. It is most likely that the pathogen population was selected for host genotype-specific virulence. These were designated as Path 1 (from NHB 3), Path 2 (BJ 104), Path 3 (MBH 110). Path 4 (852B) and Path 5 (used as a control from a mix of NHB 3 and 7042S from the downy mildew nursery).

Host genotypes

Fourteen pearl millet genotypes (inbreads and hybrids) with varying levels of resistance to downy mildew were used (Table 1) for inoculation with pathotypes. Seeds of these genotypes were obtained from genetic stocks of breeders and pathologists at the IAC. Seeds were surface-sterilized with 2% NaOCl for 4-5 min, washed thoroughly with sterilized distilled water and dried at room temperature (approximately 25° C) prior to sowing. The experiment was organized in a factorial, randomized complete block design in a greenhouse. There were four replications with about 60 seedlings per replication (30 seedlings in each of two pots). The experiment was repeated once.

Disease assessment and statistical analysis

In the context of this study, we defined 'virulence' as a qualitative measure of the relative capacity of an isolate of *S. graminicola* to infect a specific pearl millet genotype and 'aggressiveness' as a quantitative measure of the disease causing potential of an isolate and was calculated as disease incidence. In heterogeneous populations, quantitative differences in levels of virulence can be due to differences in frequency of virulence genes with qualitative effects. To determine this, we calculated virulence index (disease incidence x latent period^{-1}) to quantify the virulence level of the isolates as applicable to a heterogeneous host-pathogen system.

Infected seedlings were counted in each pot 2 weeks after inoculation and disease incidence was calculated. Latent period (the time in days between inoculation and sporulation) was recorded when first typical downy mildew symptom and sorulation became visible on the infected seedlings.

Percentage disease incidence data were arcsin transformed before subjecting these to analysis of variance (ANOVA) using the GENSTAT ANOVA procedure for factorial experiments (GENSTAT, 1986). Disease incidence data from the two experimental runs were examined for homogeneity using F-test of significance before pooling them for calculating virulence and aggressiveness. Least square differences were computed for comparing host genotype x pathotype interaction means for various parameters.

RESULTS

All five pathotypes were avirulent on two host genotypes (IP 18292 and IP 18293), but were virulent on the remaining 12 genotypes (Table 1). The pathotypes differed significantly in their aggressiveness on different host genotypes causing disease incidence in the range of 1 to 89%. The two highly susceptible genotypes, 7042S and 7042S-11, had uniformly high disease incidence and thus all the pathotypes were uniformly highly aggressive. On other genotypes, however, the incidence was highly variable within and across pathotype-genotype combinations.

The pathotypes also varied significantly for latent period, ranging from 6 to 12 days in different host genotype x pathotype combinations (Table 1). Host genotypes (704S and 7042S-11) highly susceptible to all five pathotypes had latest period 6 days while the highly resistant genotype 7042R had between 8 and 11 days. Generally, the pathotypes with specific virulence to its host genotype had significantly lower latent period (6-8 days) than those with non-specific virulence. There were variations in latent period in different host genotype-pathotype comibnations in the two experimental runs, but the pattern remained unchanged.

Table 1. Downy mildew disease incidence (DI)[a] and latent period (LP)[b] of five pathotypes of *Sclerospora graminicola* on 14 pearl millet genotypes

Genotype	Downy mildew reaction[c]	Path 1 (NHB 3)		Path 2 (BJ 104)		Path 3 (MBH 110)		Path 4 (852B)		Path 5 (NHB3+7042S)	
		DI	LP	DI	LP	DI	LP	DI	LP	DI	LP
IP 18292	R	0	-	0	-	0	-	0	-	0	-
IP 18293	R	0	-	0	-	0	-	0	-	0	-
7042R	R	13	8	12	10	1	11	9	11	13	10
P 310-17	R	20	8	8	9	8	9	7	12	12	[illegible]
P 7-4	R	19	8	17	9	8	8	16	10	29	7
MBH 110[d]	R	7	8	2	8	54	8	7	8	6	9
700651	R	56	7	40	7	10	8	30	7	29	8
ICMP 85410	R	33	9	31	7	46	7	28	7	33	8
852B	S	62	7	62	6	5	6	49	6	42	7
NHB 3[d]	S	75	6	67	6	78	6	9	6	60	6
5141B	S	62	6	65	6	78	6	26	7	62	6
BJ 104[d]	S	76	669	6	77	6	9	7	6	2	6
7042S	S	68	6	70	6	72	6	64	6	62	6
7042S-11	S	89	6	73	6	87	6	73	6	57	6

LSD (P<0.001) for pathotype x host genotype means: for DI 4.98, for LP = 0.60.

[a]Arcsin transformed values of percentage of infected seedlings, based on the average of two experimental runs with 60 seedlings in each replication.

[b]Time in days between inoculation and appearance of symptom and sporulation based on the mean of two experimental runs.

[c]Based on field reaction for 3-5 years at several locations in India.

[d]Pedigree of hybrids: MBH 110 (MS2 x PL2); NHB 3 (Tift 23A × J104); BJ 104 (5141A × J104).

Clear differential interactions were observed among eight host genotypes for the five pathotypes. Path 1 was highly aggressive (75% incidnece) on NHB 3 (Tift 23A x J-104), its own selection host and on 5141B and BJ 104 (5141A x J104) the lines related to NHB 3, and it was least aggressive (7% incidence) on MBH 110 (MS2 x PL 2). Similarly, Path 2 was highly aggressive on BJ 104 (69% incidence) and NHB 3 (67% incidence), and 5141B (65% incidence) which is related to BJ 104, and was less aggressive on MBH 110 and P 310-17. Path 3 was moderately aggressive (54% incidence) on its selection host MHB 110, highly aggressive on NBH 3, 5141B and BJ 104, and less aggressive on 852B and 700651. Path 4 was less aggressive on MBH 110, NHB 3, and BJ 104, but highly aggressive on its selection host 852B. Path 5, the field population was least aggressive on

Table 2. Analysis of variance for aggressiveness and virulence index

Source of variation	df	MS	
		Aggressiveness	Virulence index
Pathotype (P)	4	6235.39***	230.93***
Host genotype (G)	11	21947.29***	824.16***
Experiment (E)	1	116.47	323.20***
Rep/experiments	6	14.43	0.63
P × G	44/43	2064.70***	65.05***
P × E	4	650.10***	50.42***
G × E	11	62.30**	26.29***
P × G × E	44	83.02***	5.79***
Pooled error	354/297	25.87	0.82

and * Significant at $P < 0.01$ and $P<0.001$, respectively.

MBH 110, moderately aggressive on P 7-4 and 700651, and highly aggressive on its selection host NHB 3 and related genotypes 5141B and BJ 104.

The ANOVA for virulence and aggressiveness showed highly significant ($P<0.001$) effects of pathotype, host genotype and their interaction (Table 2). Significant effects were also observed for pathotype x experiment, genotype x experiment and pathotype x genotype x experiment interactions, but the mean sum of squares for these interactions were much less than that of the pathotype x genotype interaction. Host genotype seems to have much larger effects than pathotype or pathotype x genotype interactions.

In certain host genotype x pathotype combinations, the patterns of aggressiveness were quite different from that of virulence index (VI), which is a measure of quantitative virulence. In general, all pathotypes were less virulent (<2.52 VI) on resistant genotypes 7042R and P 310-17, and more virulent (9.74-15.92 VI) on the susceptible genotypes 7042S and 7042S-11 (Table 3). Path 1 was most virulent (15.56 VI) on its selection host NHB 3 and least virulent (1.28 VI) on MBH 110. Similarly, Path 2 was most virulent (12.66 VI) on BJ 104 and least virulent (0.05 VI) on MBH 110. Path 3 was highly virulent (8.21 VI) on its selection host MBH 110, but was more virulent (14.12-14.40 VI) on NHB3, 5141B and BJ104, and least virulent (1.24 VI) on 8528. Path it was highly virulent (8.21 VI) on its selection host 852B, but was contrastingly less virulent on NHB 3, 5141B, BJ 104 and MBH 110. Path 5, as expected, was more virulent on its selection hosts than on others. All pathotypes, except Path 4, although highly aggressive on 5141B (Table 1), were less virulent because of prolonged latent period. Significant effects of pathotype, host genotype, experiment, pathotype x genotype, pathotype x experiment, genotype x experiment and pathotype x genotype x experiment interaction were found for VI (Table 2). Among the interaction effects, pathotype x genotype was more pronounced and variation between two experimental runs were also evident due to highly significant variance for experiment.

Discussion

Highly significant effects of pathotype x host

Table 3. Virulence index[a] (disease incidence × latent period^{-1}) of five pathotypes of *Sclerospora graminicola* on 12 pearl millet genotypes

Genotype	Path 1 (NBH 3)	Path 2 (BJ 104)	Path 3 (MBH 110)	Path 4 (852B)	Path 5 (NHB3+7042S)
7042R	1.56	1.79	2.52	0.19	1.56
P 310-17	2.27	1.50	1.06	0.78	1.46
P 7-4	2.48	2.37	1.35	2.34	4.05
MBH 110	1.28	0.05	8.21	1.79	1.13
700651	7.46	6.26	1.25	4.86	3.65
ICMP 85410	3.75	4.72	7.03	4.05	4.33
852B	8.88	11.41	1.24	8.21	5.73
NHB 3	15.56	12.25	14.32	1.78	9.70
5141B	10.71	12.02	14.40	4.24	11.19
BJ 104	13.91	12.66	14.12	1.45	10.72
7042S	13.11	12.89	13.25	11.79	10.12
7042S-11	15.87	13.41	15.92	13.41	9.74

LSD (P<0.001) for pathotype × host genotype means = 0.89

[a]Based on the mean of two experimental runs.

genotype interactions for virulence and aggressiveness indicate, according to Vanderplank (1984), the occurrence of host-pathogen specificity in the pearl millet-downy mildew pathosystem. In general, each pathotype was highly aggressive and highly virulent (as expressed by VI) on its selection host and uniformly susceptible genotypes. However, Path 3 was relatively less virulent on its selection host MBH 110 than on NHB 3, 5141B and BJ 104, indicating the presence of virulence gene(s) non-specific to these genotypes. All five pathotypes were avirulent on IP 18292 and IP 18293; less virulent on 7042R, P 310-17 and P 7-4; moderately virulent on ICMP 85410; and highly virulent on 7042S and 7042S-11 indicating the range of resistance/virulence genes available in the host genotypes and pathotypes. Generally, VI and aggressiveness were closely related (P<0.001), but some exceptions occurred involving ICMP 85410. All five pathotypes were moderately aggressive on ICMP 85410 (28-46% incidence), but they had relatively lower VI (3.75-7.03) more typical of a resistant host genotype. This suggests that ICMP 85410 may have non-pathotype-specific, partial resistance that could be durable. The lower VI of ICMP 85410 were due to relatively longer latent period (7-9 days).

Results indicate that 700651 and 852B probably share a common resistance gene(s) and that BJ 104 and NHB 3 have a common resistance gene(s) different from those in 700651 and 852B. Host genotypes IP 18292 and IP 18293 which showed no infection with any of the five pathotypes seem to possess different or more resistance genes. Evidences from this study and earlier findings (Appadurai *et al.*, 1975; Ball, 1983; Ball and Pike, 1984; King *et al.*, 1989; Thakur and Shetty, 1993) are supportive of evolution of virulence factor(s)

specific to host resistance factor(s) in this pathosystem, and provide the basis for future research. In a recent study on mapping quantitative trait loci (QTL) for downy mildew resistance in pearl millet, Jones *et al.* (1995) have shown QTLs of large effects contributing towards variation in resistance in different pearl millet genotypes against the pathogen populations from Africa and India. There were no QTLs that were effective against all four pathogen populations under test indicating the existence of host genotype-specific resistance as a major mechanism of resistance in the pearl millet-downy mildew system.

Breeding and selection for downy mildew resistance in pearl millet, therefore, should be based on multiple screening against existing pathotypes. Resistance in IP 18292, IP 18293, P 310-17, 7042R, and P 7-4 is likely to be more stable as these are resistant to all five pathotypes tested, while ICMP 85410 might possess more resistance genes and could prove a good source of durable resistance. Further studies on genetics and the biochemical basis of host-pathogen interactions are needed for better utilization of downy mildew resistance in pearl millet.

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