Efficiency of Pathotype Mixture in Screening for Downy Mildew Resistance in Pearl Millet

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Introduction

Sclerospora graminicola (Sacc.) Schröet., the causal agent of downy mildew disease in pearl millet [*Pennisetum glaucum* (L.) R. Br.], has an inherent mechanism to develop variability because of heterothallism (Michelmore et al. 1982). The pathogen survives through sexually produced oospores, which are genetic recombinants, and the pathogen populations can, therefore, be highly variable and adaptable in different environments. The existence of variability in *S. graminicola* has been reported and different host-specific pathotypes have been identified (Thakur and Rao 1997; Thakur 1999; Thakur et al. 2003). Development of improved breeding lines as potential parental lines (A-, B- and R-lines) of hybrids with downy mildew resistance has been the major research focus at ICRISAT-Patancheru, India. These breeding lines are routinely screened against individual pathotypes in succession to identify those with resistance to single or multiple pathotypes (Thakur et al. 2001). Frequently, a question is asked "why cannot pearl millet lines be screened against a mixture of pathotypes instead of individual pathotypes in order to identify resistance against multiple pathotypes, and therefore, make the screening system more time- and resource-effective?" To address this question, we studied the efficiency of a mixture of two diverse pathotypes of *S. graminicola* in identifying pearl millet lines with resistance to either or both.

Materials and Methods

Downy mildew pathotypes. Two important and diverse pathotypes of *S. graminicola*, Sg 150 from Jalna (Maharashtra state, India) and Sg 212 from Durgapura (Rajasthan state), which are often used in the screening of advanced pearl millet breeding lines at ICRISAT, were used both individually and as a mixture (equal concentrations of sporangia and equal volumes of inoculum). These pathotypes were maintained through asexual generation on seedlings of their respective susceptible lines (Sg 150 on 834B and Sg 212 on ICMP 451) under greenhouse conditions at ICRISAT-Patancheru (Thakur et al. 2001).

Pearl millet lines. A set of 14 pearl millet lines, including some that are known for their differential reactions to the two pathotypes, and two susceptible controls were used in the experiment. The experiment was conducted in a completely randomized design (CRD) with three treatments of the pathogen and 14 pearl millet lines in six replications (one pot per replication with 35–40 seedlings per pot), and it was repeated once.

Inoculum and inoculation. The sporangial suspensions (1×10^6 mL^{-1}) of the two pathotypes individually and in mixture were used to spray-inoculate the pot-grown seedlings of pearl millet lines at the coleoptile to one-leaf stage using an atomizer. The inoculated seedlings were incubated in the dark at 20±1°C for 16 h in the inoculation chamber, and the pots were then shifted to greenhouse benches (25±1°C and >95% RH provided by overhead foggers) for disease development.

Data recording and analysis. The latent period (the number of days between inoculation and symptom appearance and sporulation on 50% of the total infected plants), disease incidence (percentage of seedlings infected) and virulence index (VI = disease incidence × latent period^{-1}) were recorded as per Thakur and Rao (1997). The latent period was recorded from the 4th day after inoculation until the 14th day, and the number of days required to infect 50% of the total infected plants per pot was considered to obtain uniform data for pathotypes across pearl millet lines. Downy mildew incidence in each pot was recorded 14 days after inoculation.

The data on disease incidence, latent period and VI were subjected to analysis of variance (ANOVA) to determine the effects of different factors and their interactions. The LSD values were computed to compare the differences between disease incidence and latent periods and SEm values to compare the mean VI caused by individual pathotypes and their mixture on pearl millet lines. Since there were no significant differences in the error MS in the two experimental runs, ANOVA was conducted on pooled data sets.

Results and Discussion

Effect on downy mildew incidence. The ANOVA indicated highly significant (P <0.001) effects of pathotypes, pearl

millet lines and their interactions on downy mildew incidence (Table 1). The two pathotypes, Sg 150 and Sg 212, and their mixture caused varying levels of downy mildew incidence ranging from 0% to 100% on the 14 pearl millet lines (Table 2). There was no significant difference in the downy mildew incidence caused by the two pathotypes and their mixture on seven lines (P 310-17, 7042R, IP 18292, 863B, ICMB 03999, S 2003-188 and 7042S), of which four (P 310-17, IP 18292, 863B and ICMB 03999) were highly resistant (0–6% incidence), two (S 2003-188 and 7042S) highly susceptible (94-100% incidence) and one (7042R) moderately susceptible (36–38% incidence) to both pathotypes and their mixture (Table 2). Of the three lines (834B, 843B and ICMP 451) which were highly susceptible to either of the pathotypes, the downy mildew incidence caused by the pathotype mixture was similar to the one obtained by the more virulent pathotype (Sg 150). On the remaining four lines (P 7-4, 700651, 852B and IP 18293) Sg 150 appeared more virulent than Sg 212, the disease incidence levels caused by the pathotype mixture, except on IP 18293, were also closer to those caused by the more virulent pathotype Sg 150.

Pathotype Sg 150 showed host-specific virulence on P 7-4, 700651, 852B and 834B (30–98% incidence) whereas Sg 212 showed host-specific virulence on ICMP 451 and 843B (99–100% incidence). In such cases, where a pathotype had host-specific virulence and caused higher disease incidence, the mixture behaved like the more virulent pathotype. In other cases, where the two pathotypes were equally virulent, the disease incidence caused by the mixture was toward the mean of the two. Thus, disease incidence caused by the mixture indicates the dominance of the more virulent pathotype.

Effect on latent period. The ANOVA showed highly significant ($P<0.001$) effects of pathotypes, pearl millet lines and their interactions on latent period (Table 1). The two pathotypes (Sg 150 and Sg 212) and their mixture showed latent period in the range of 5 days to 12 days (Table 2). The pathotype Sg 150 had the minimum latent period of 6.3–6.6 days on 7042S, ICMP 451 and S 2003-188 whereas, Sg 212 and the mixture had 5.0–5.3 days and 5.1–5.5 days, respectively. The longest latent period (11.8 days) for Sg 212 was recorded on 852B, while for Sg 150 and the mixture it was 12 days on ICMB 03999. The latent period values on P 7-4, 700651, 863B and IP 18293 by individual pathotypes and their mixture were similar (Table 2). It was again similar on 843B, IP 18292, S 2003-188 and ICMP 451 by Sg 212 and the pathotype mixture, and also on ICMB 03999 by Sg 150 and the mixture. These results indicate that there was no specific trend of increase or decrease in the latent period by the pathotype mixture compared to single pathotypes on most of the pearl millet lines.

Effect on virulence index. Similar to downy mildew incidence and latent period, highly significant ($P<0.001$) effects of pathotypes, pearl millet lines and their interactions were noted for VI (Table 1). The maximum VI was observed on the highly susceptible line 7042S, while ICMB 03999 recorded the minimum both by individual pathotypes and their mixture (Fig. 1). The VI values of Sg 212 and the mixture were comparable on 843B, IP 18293, S 2003-188, ICMP 451 and 7042S. Similarly, the VI values of Sg 150 and the mixture were comparable on 7042R, IP 18292 and 863B. However, there were large differences in the VI of the single pathotypes. The VI values due to the mixture were closer to those by the more virulent type. These results thus indicated that there were no additive effects in virulence by the mixture of pathotypes.

Based on both downy mildew incidence and VI, it appeared that the pearl millet lines that were highly susceptible to either or both pathotypes were also highly susceptible to the pathotype mixture, and the lines that were not highly susceptible to either of the pathotype had average susceptibility to the mixture. In both cases, the

Table 1. Analysis of variance for downy mildew incidence (DM inc), latent period (LP) and virulence index (VI) caused by two pathotypes and their mixture on 14 pearl millet genotypes.

		MS		
Source of variation	df	DM inc	LP	VI
Replications	5	92.52	1.37	2.24
Pathotypes	2	2252.79***	11.28***	46.57***
Lines	13	53275.37***	142.87***	1779.48***
Pathotypes × lines	26	3261.87***	12.58***	113.30***
Residual	457	41.70	1.87	2.75

***Significant at $P<0.001$.

Table 2. Downy mildew (DM) incidence and latent period (50%) caused by two pathotypes of *Sclerospora graminicola* and their mixture on a set of pearl millet lines in a greenhouse.

	DM incidence (%)[1]			Latent period$_{50\%}$ (days)[1]		
Pearl millet line	Sg 150	Sg 212	Sg 150+Sg 212	Sg 150	Sg 212	Sg 150+Sg 212
P 7-4	34	4	14	8.6	8.5	8.0
P 310-17	6	1	2	10.7	7.0	11.7
700651	30	13	17	8.8	8.6	8.5
7042R	36	38	37	7.4	6.2	6.8
852B	46	3	19	8.8	11.8	8.1
834B	98	23	86	7.1	7.0	8.6
843B	76	100	98	6.7	5.4	5.3
IP 18292	4	2	2	9.5	10.3	10.5
IP 18293	19	7	8	8.1	8.9	9.1
863B	3	2	3	9.5	9.8	9.0
ICMB 03999	1	0	2	12.0	–[2]	12.0
S 2003-188	96	99	99	6.6	5.3	5.5
ICMP 451	40	99	95	6.4	5.0	5.2
7042S	94	100	97	6.3	5.1	5.1
Mean	41	35	41	8.3	7.5	8.1
LSD (P<0.05)	6.1	4.7	4.9	1.20	1.17	0.95

1. Mean of 2 runs, 6 replications per run.
2. No infection recorded.

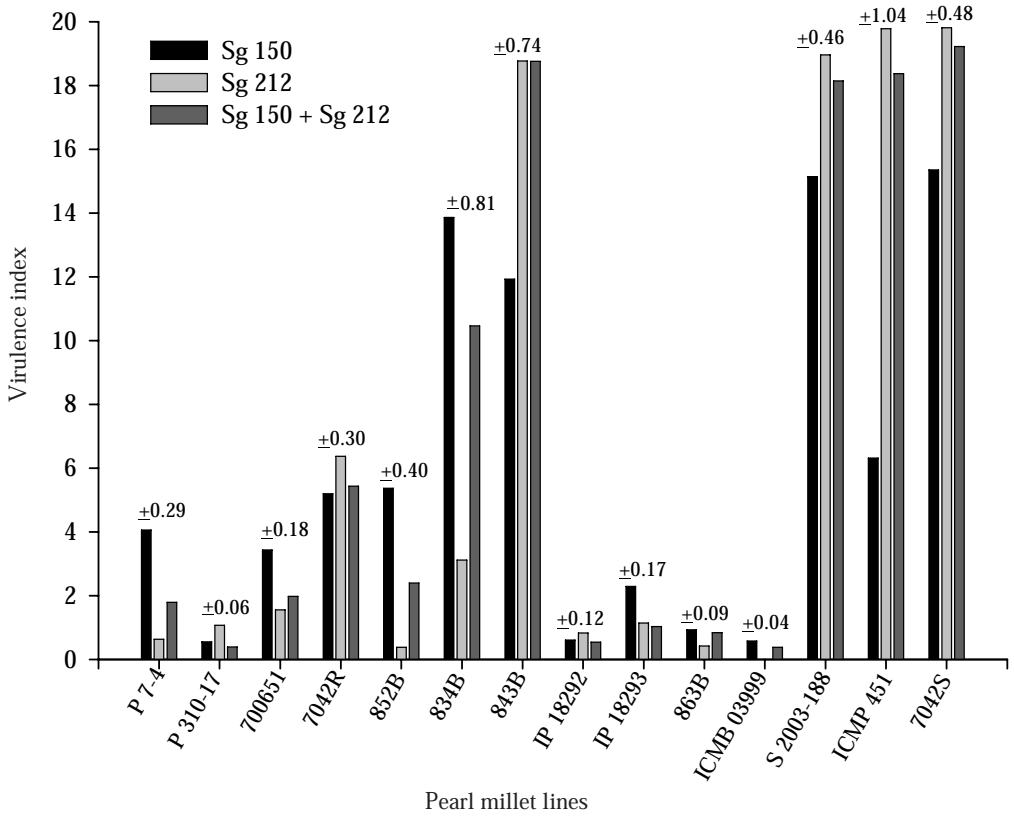

Figure 1. Virulence index (downy mildew incidence × latent period^{-1}) caused by two pathotypes of *S. graminicola* and their mixture on 14 pearl millet lines.

use of the pathotype mixture would serve the purpose of discarding lines that are highly susceptible and thus could economize on resources used for screening. However, the time and effort required to multiply inoculum of individual pathotypes, preparing the right mixture of sporangial suspension and the number of pearl millet lines to be screened need to be considered before using the pathotype mixture in screening breeding lines. In the pearl millet–*S. graminicola* pathosystem, which is highly dynamic and where virulence evolution is highly host-dependent (Thakur et al. 1992; Thakur 1999), resistance screening against specific virulent pathotypes would always be more important for targeted resistance breeding than screening against a mixture of pathotypes.

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