Genetic Resistance of Pearl Millet Male-Sterile Lines to Diverse Indian Pathotypes of *Sclerospora graminicola*

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ABSTRACT

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Single-cross F_1 hybrid cultivars based on cytoplasmic-nuclear male-sterility (CMS) system have contributed significantly to increasing productivity of pearl millet (*Pennisetum glaucum*). Genetic resistance to downy mildew (*Sclerospora graminicola*) in parental lines is critical for successful commercial cultivation of a hybrid cultivar. In this study, 46 genetically diverse male-sterile lines (A-lines), including 42 test A-lines, four control A-lines, a commercial hybrid, and a highly susceptible line, were evaluated in disease nurseries at four diverse locations in India and compared with pathotype isolates from the same locations under greenhouse environments. Variability in downy mildew incidence (0 to 100%) due to genetic differences among lines, among pathotypes, and that due to line × pathotype interaction were all highly significant ($P < 0.001$). In the field experiment, eight of the 42 test A-lines, including 841A (control), that recorded ≤10% disease incidence, were identified as resistant compared with 84 to 100% incidence on the control susceptible line 7042S. Resistance in eight of these test A-lines (863A, ICMA 88004, -94333, -98222, -98111, -92777, and -96666) and 841A was confirmed against the four pathotypes in greenhouse experiments. Cluster analysis of downy mildew incidence data from field and greenhouse experiments, using the Euclidian distance, classified the 48 lines into four distinct groups with the above eight A-lines in the resistant group. These resistant A-lines would be useful in the development of F_1 hybrids with stable resistance to diverse pathotypes of downy mildew in India.

Downy mildew, caused by *Sclerospora graminicola* (Sacc.) J. Schröt), is a serious disease of single-cross F_1 hybrid cultivars of pearl millet (*Pennisetum glaucum* (L.) R. Br.). Single-cross F_1 hybrid cultivars based on an A_1 cytoplasmic-nuclear male-sterility (CMS) system have contributed significantly in increasing productivity of pearl millet in India (2,7,18). Since the release of the first series of pearl millet F_1 hybrids in India during the late 1960s, downy mildew, hitherto known as a minor disease, became a disease of significant economic importance (10,11,12,25). The first widely cultivated commercial hybrid HB 3, developed on Tift 23A, was released in 1968, and the first epidemic of downy mildew occurred in 1971, causing a severe grain yield loss (12,15,16). Since then, a number of hybrids have succumbed to downy mildew and have been withdrawn from cultivation (15,22). Although the A_1 cytoplasm of male-sterile lines (A-lines) has been shown not to be involved in susceptibility to downy mildew (1,27), the genetic uniformity of single-cross F_1 hybrids provides little barrier, if any, to the pathogen in rapidly adapting to the new cultivar and overcoming the host resistance compared with genetically heterogeneous open-pollinated varieties (18,23). High harvest index, early maturity, and uniform crop stature of F_1 hybrid cultivars have attracted farmers, and currently about 55% of the total 10 million ha under pearl millet cultivation in India is being grown with hybrid cultivars. During the past 20 years, private seed companies, in association with the Pearl Millet Improvement Program of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Indian Council of Agricultural Research (ICAR) have played a significant role in extending hybrid cultivars to farmers. Today, more than 50 hybrids are being cultivated in India (18). The downy mildew pathogen has evolved rapidly to keep pace with changing cultivars. Evidence exists of evolution of several races or pathotypes specific to popular hybrid cultivars in India (14,21,22,23). In our recent field surveys in states of Maharashtra, Rajasthan, and Gujarat, disease incidence was 80 to 100% on a few hybrids in some farmers' fields (23), accounting for a considerable yield loss. Results of the International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN) have provided further evidence for the existence of variable pathotypes within and between countries in Asia and Africa (9). Circumstantial evidence exists for the emergence of a new virulent pathotype specific to an F_1 hybrid cultivar when it has been grown consecutively for 3 to 4 years in the same fields.

ICRISAT has a major research focus on developing parental lines, especially diversifying the genetic base of A-lines, which are disseminated to public organizations and private seed companies for use in developing F_1 hybrid cultivars (3,8). Such hybrids have wider adaptation and higher grain yield potential. Our objective in this study was to evaluate the recently developed A-lines and identify those having resistance to diverse pathotypes of *S. graminicola* that exist in India. Such resistant A-lines, in combination with resistant male parents, would lead to the development of F_1 hybrids that will have broad-based resistance against diverse pathotypes and hence will likely be more stable for downy mildew resistance.

MATERIALS AND METHODS

Male-sterile lines. Forty-six male-sterile lines (A-lines) consisting of 42 test A-lines and four control A-lines (81A, 841A, 843A, and 852A) along with a commercial hybrid (MBH 110) and a highly susceptible inbred line (7042S) were included in this study (Table 1). All the A-lines carry A_1 cytoplasm except ICMA 97555, which carries A_4 cytoplasm (18). The maintainer lines (B-lines) of all these A-lines were developed at ICRISAT, Patancheru. A majority of these was bred by crossing two commercial B-lines (81B and 843B) bred during the early 1980s with a diverse range of improved germ plasm providing sources of genetic diversity and downy mildew resistance. Maintainers of three A-lines (863A, ICMA 88004, and ICMA 98222) were developed by inbreeding and direct selection in the *Iniadi* landrace germ plasm. Maintainers of some of the A-lines produced during the late 1980s from earlier selection programs (e.g., ICMB 88006, ICMB 89111, ICMB 88004) that became commercial during subsequent years were involved in the second round of crossing and pedigree breeding of B-lines. The set of A-lines included in this study represents the widest range of diversity in A-line collections anywhere in the world. Among the four A-lines that were used as controls, 81A is the first A-line developed at ICRISAT, Patancheru, and it has been among the most widely used A-lines for breeding commercial hybrids in India. 841A has

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shown high degrees of resistance to downy mildew across locations, 843A is highly susceptible to most pathotypes, and 852A has shown differential reaction with specific susceptibility to the Mysore pathotype (21). Two additional entries that were included as controls in this study are an F_1 hybrid (MBH 110) that has shown differential reaction with specific susceptibility to the Jalna pathotype and a universal susceptible line (7042S).

Pathotypes of *S. graminicola*. Four pathotypes of *S. graminicola* from Patancheru (Sg 153), Mysore (Sg 048), Durgapura (Sg 004), and Jalna (Sg 150) were maintained through asexual generations on pot-grown seedlings of different pearl millet lines in polyacrylic isolation chambers (45 × 45 × 45 cm) in a greenhouse at ICRISAT, Patancheru. Initial asexual inoculum of each pathotype isolate was established by growing pearl millet seedlings in pot soil infested by the respective oospores. Patancheru pathotype isolate was obtained from NHB 3, Durgapura pathotype from a susceptible local land race cultivar, Mysore pathotype from 852B, and Jalna pathotype from MBH 110. Patancheru and Durgapura pathotype isolates were maintained on 7042S, Mysore pathotype on 852B, and Jalna pathotype on MBH 110 through asexual generations for this study. The isolates from Durgapura and Patancheru were maintained on the universal susceptible line 7042S because of nonavailability of pure seeds of the host lines from which these isolates were collected. These isolates were maintained for three to four asexual generations on seedlings in greenhouse isolation chambers before being used in this study.

Field evaluation. Seeds were sown in downy mildew nurseries at four locations in four states of India during the rainy season 1998. These were Patancheru (17.53°N) in Andhra Pradesh, Mysore (12.30°N) in Karnataka, Durgapura (26.92°N) in Rajasthan, and Jalna (19.83°N) in Maharashtra. Disease nurseries were created by incorporating diseased-leaf tissues containing oospores from the previous season's crop (16) supplemented with an infector-row system (26).

Disease pressure in nurseries was considered adequate for downy mildew evaluation when a susceptible control line had above 70% disease incidence. Each line was grown in 2 rows, 4 m long, in two or three replications in a randomized complete block design. Adequate fertilization and irrigation were provided to raise a good crop. Downy mildew was rated twice, at 30 and 60 days after seedling emergence, by counting the total plants and number of infected plants in each plot. The numbers of total plants per plot per replication were from 100 to 120. Since there was an increase in the number of infected plants in some lines at 60 days compared with 30 days, the 60-day data were used for final analysis.

Greenhouse evaluation. Pearl millet seeds were sown in plastic pots (15 cm diameter) filled with autoclaved potting mix of soil, sand, and farmyard manure (2:1:1, vol/vol/vol). There were three replications, each with two pots and having a total of about 100 seedlings per entry per replication. Three days after sowing, all the emerged seedlings in each pot were counted. Seedlings that emerged after inoculation were disregarded. Sporangial suspensions (1×10^6 spores ml^{-1}) were prepared from the pot-grown pearl millet

Table 1. Parentage of pearl millet male-sterile lines evaluated for resistance to downy mildew using diverse Indian pathotypes of *Sclerospora graminicola*

Male-sterile line	Parentage of maintainer line (B-line)
863A	Togo-13-4-1
ICMA 87001	{Tift 23D2B(I) × (J 1623 × 3/4EB-96-1-7)}-3-B
ICMA 88004	Togo-11-5-2 selection
ICMA 88006	{(81B × SRL 53-1) × 843B}-30-2-B
ICMA 89111	{843B × (GNS × SS-48-40-4)-1-9-8}-30-B-B-1
ICMA 91222	(26B × 81B)-4-1-2
ICMA 91444	{843B × (Boudama-481 × Ankoutess-2)-4}-2-B
ICMA 91666	{843B × (J 1623 × 3/4 EB-96-1-10)}-92-2-1
ICMA 91777	{843B × (J 1623 × 3/4 EB-96-1-10)}-5-2
ICMA 92111	(81B × 843B)-11-1-1-B
ICMA 92333	(843B × 81B)-30-1-1
ICMA 92444	(843B × ICMPS-1500-7-4-1-6)-23-1-B-1-4
ICMA 92666	{ICMPES-34 × (843B × ICMPES-34)}-155-4-2
ICMA 92777	{843B × (ICMPS 500-4-4-3 × ICMPS 1800-3-1-2-C3-4)}-7-1-3
ICMA 92888	(843B × ICMPS 900-9-3-2-2)-41-2-6-2-2
ICMA 93111	{(81B × SRL 53-1) × 843B}-30-1-1
ICMA 93222	(26B × 834B)-11-2-B-B
ICMA 93333	(843B × ICMPS 900-9-3-8-2)-21-8-4
ICMA 94111	[(ICMB 89111 × ICMB 88002) × {(81B × SRL 53-1) × 843B}-3-5-2-1-16 × IP 9402-2-1-1-4]-31
ICMA 94222	[{{843B × (843B × 700651)}-11-1-2-B × 1163B} × (ICMB 89111 × ICMB 88004)]-3-3
ICMA 94333	(843B × Togo plot 26-1)-27-B
ICMA 94444	(843B × 405B)-4-B
ICMA 94555	[{{843B × (843B × 700651)}-11-1-2-B × 1163B} × (ICMB 89111 × ICMB 88004)]-5-3
ICMA 95111	[{843B × (GNS × SS-48-40-4)-1-9-8}-30-B-B-1 × {843B × (843B × 700651)-11-1-2-B}]-39-B
ICMA 95222	[{843B × (GNS × SS-48-40-4)-29-7-4-B} × (843B × ICMPES-29)-23-2-3]-16-B
ICMA 95333	[{(B282 × S10B-38)-35 × Togo-29-2-2}-53 × {843A × {843B × (B282 × 3/4EB-100)-11-9-2}}]-60-29-1
ICMA 95444	(81-1164 DB/85-1856LR-16-B × 843DMR1)-14-6-3
ICMA 95555	DMR1 S2-96-2-3-4
ICMA 96111	(843B × 81B)-58-1-1-1
ICMA 96222	[{26B × (81B × SRL 50-1)}-1-1-2 × 852B]-69-1-1
ICMA 96333	[{{843B × (843B × 700651)-11-1-2-B}× 1163B} × (ICMB 89111 × ICMB 88005)]-5-2-2
ICMA 96444	(SPF3/S91-933 × SPF3/S91-3)-4-2-3-1
ICMA 96555	(SPF3/S91-5 × ARD early bulk)-2-3-3
ICMA 96666	(SPF3/S91-327 × SPF3/S91-5)-6-2-3
ICMA 97111	HTBC-48-B-1-1-1-1
ICMA 97222	{(ICMB 88006 × ICMB 88005) × (ICMB 89111× ICMB 88004)}-28-2-B
ICMA 97333	(ICMB 89111 × ICMB 88004)-9-2-6-3-3-2-B
ICMA 97444	DMR1 S2-133-1-2-4-B
ICMA 97555	(SPF3/S91-5 × ARD early bulk)-2-3-2
ICMA 98111	HTBC-HS-27-1-1-1
ICMA 98222	ARD-288-1-10-1-2 (RM)-5
ICMA 98666	(ICMB 89111 × IPC 1466)-21-1-3-6-B-5
81A	Mutation-induced downy mildew resistant version of Tift 23D2A
841A	Downy mildew resistant selection of 5141A
843A	Selection from KSU line AKM 2068
852A	(MC-103 × Serere 17B-12-2)-1
MBH 110	MAHYCO hybrid
7042S	Selection from a landrace from Chad
Decoding of abbreviations used in pedigree of some B-lines	
843DMR1	{843B × (843B × 700651)}-11-1-2-B
DMR1	[{843B × (843B × 700651)}-11-1-2-B]-96-2-3-4
SPF3/S91-933	[{{843B × (GNS × SS-48-40-4)-1-9-8}-30-B-B-B} × (842B × 3/4EB-11-9-2-1)-44-5-1]-10
SPF3/S91-3	[{{{843B × (843B × 700651)}-11-1-2-B} × 81-1163B} × (ICMB 89111 × ICMB 88005)]-3-3
SPF3/S91-5	[{{{843B × (843B × 700651)}-11-1-2-B} × 81-1163B} × (ICMB 89111 × ICMB 88005)]-5-3
SPF3/S91-327	{(ICMB 88006 × ICMB 88005) × (ICMB 89111× ICMB 88004)}-7

seedlings infected with each isolate of the four pathotypes. Seedlings (one- to two-leaf stage) were spray-inoculated by a hand-held atomizer in the inoculation chamber and covered with polyethylene sheet to provide >95% relative humidity necessary for infection. Inoculated seedlings were incubated in the dark at 20°C. The pots were moved after 24 h to a greenhouse at 25 ± 2°C and arranged in a completely randomized block design. Downy mildew infection was recorded 2 weeks later by counting the number of diseased seedlings in each pot. Numbers of total and diseased seedlings were used to calculate the percent disease incidence. The experiment was repeated once with all four pathotypes.

Data analysis. Analysis of variance of downy mildew incidence data was done on both original and arcsine transformed scales using a fixed-effects model. Since the results of the analyses on both scales were similar, the mean incidence data were presented on the original scale. The analysis was done using GENSTAT statistical package (Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK). The field trial at each location was laid out in an RCBD, and observations on each plot were made at two times. Thus, the two time points were nested within each plot. Therefore, in order to compare and determine the effect of time (30- and 60-day data) on disease incidence, data from each location were analyzed as a split-plot trial with two time points as subplots. The time × line interaction effects were significant ($P < 0.05$) at all locations. However, since the interactions were of non-crossover types, only the 60-day data were used for final analysis. Downy mildew incidence data sets both from field and greenhouse tests were subjected to an average linkage cluster analysis using Euclidian distance as dissimilarity measure to determine the association among the A-lines and classify them into major resistant and susceptible groups for strategic utilization in resistance breeding.

RESULTS

Resistance in field nurseries. Downy mildew incidences recorded at 30 and 60 days in four disease nurseries at four locations were similar for most pearl millet lines except at Mysore, where there was considerable increase in disease incidence in 21 of the 48 lines at 60 days (30-day data not provided). Analysis of variance for downy mildew incidence across four locations over two times indicated highly significant ($P < 0.001$) effects of location (pathotype), pearl millet lines, pathotype × line interaction, time point, pathotype × time, line × time, and pathotype × line × time ($P < 0.004$) (Table 2).

Downy mildew pressure was high in all four tests in disease nurseries with 84 to 100% disease incidence on the susceptible control line 7042S (Table 3). We considered a line resistant if it had ≤10% disease incidence and moderately resistant to highly susceptible if it had 11 to 100% disease incidence. Of the 42 test A-lines, 38 were resistant at Patancheru, 32 at Durgapura, 25 at Mysore, and 22 at Jalna. There were eight lines (863A, ICMA 88004, -92111, -92777, -94333, -96666, -97111, and -98111) that had ≤10% disease incidence across all four locations. Among the A-lines used as controls, 841A was resistant and 843A was susceptible at all four locations, while 81A was resistant at Patancheru and Mysore but susceptible at Durgapura and Jalna. MBH 110, a popular F_1 hybrid in Maharashtra until a few years ago, had high disease incidence (81%) at Jalna, while it recorded low disease incidence (13%) at Durgapura and remained resistant at Patancheru and Mysore and was identified as a differential host with high specific susceptibility to Jalna pathotype. Likewise, 852A, identified earlier as a differential host with high specific susceptibility to Mysore pathotype, recorded high disease at Mysore (79%), had low disease incidence at Jalna (24%) and Patancheru (13%), and was resistant at Durgapura. Differential disease reactions across locations were evident in many test A-lines. For instance, ICMA 98666 was susceptible at Patancheru (20% incidence) and Mysore (55% incidence), but remained resistant at the other two locations. At Jalna, there were as many as 15 test A-lines that had >20% disease incidence, and seven of these had 44 to 76% incidence.

Resistance in greenhouse evaluation. Variability in downy mildew incidence due to genetic differences among A-lines, due to pathotype, and due to line × pathotype interaction were all highly significant ($P < 0.001$) (Table 4). In both runs, the disease pressure with all four pathotypes was quite high, with 71 to 95% incidence on the susceptible control 7042S (Table 3). As in field tests, many lines were resistant (≤10% disease incidence) to each of the four pathotypes. Of the 42 test A-lines, 33 were resistant to the Patancheru pathotype (Sg 153), 29 to the Mysore pathotype (Sg 048), 28 to the Durgapura pathotype (Sg 004), and 15 to the Jalna pathotype (Sg 150). Again, differential reaction across pathotypes was evident in many lines. For instance, there were 9, 7, and 5 test A-lines that had >20% downy mildew incidence against each of the Patancheru, Durgapura, and Mysore pathotypes, respectively. More than 40% incidence was recorded in six of these A-lines against the Patancheru pathotype, in one A-line against the Durgapura pathotype and in two A-lines against the Mysore pathotype. Against the Jalna pathotype, there were 20 test A-lines that had >20% disease incidence, with 12 of these having ≥40% incidence.

Several A-lines provided differential reactions to the four pathotypes. Twelve test A-lines (863A, ICMA 88004, -88006, -92777, -94333, -95444, -95555, -96666, -97444, -97555, -98111, and -98222) recorded ≤10% disease incidence against all four pathotypes. As in field tests, 841A was resistant to all four pathotypes, while 81A and 843A were susceptible to highly susceptible to different pathotypes. Again, MBH 110 remained resistant to all pathotypes, except to the Jalna pathotype. Downy mildew resistance of seven of these A-lines (863A, ICMA 88004, -92777, -94333, -96666, -98111, -98222) out of the nine identified in the field test was confirmed in the greenhouse tests.

Resistance in both field and greenhouse evaluations. Using the average linkage cluster analysis for downy mildew incidence data from both field and greenhouse tests, the 48 lines were classified into four distinct groups (Fig. 1). These may be considered resistant (9 lines, including 841A), moderately resistant (10 lines), susceptible (12 lines, including 852A), and highly susceptible (17 lines, including MBH 110, 81A, 843A, and 7042S) groups. ICMA 96333, although classified into the resistant group by cluster analysis, had 16 and 11% incidence in the field nursery at Mysore and to Jalna pathotype in the greenhouse, respectively (Table 3).

DISCUSSION

Evaluation of recently developed genetically diverse and agronomically elite pearl millet A-lines against diverse Indian patho-

Table 2. Analysis of variance for downy mildew incidence of 48 pearl millet lines at two times (30 and 60 days) after emergence under field conditions

Source of variation	df	Mean squares[a]
Location (pathotype)	3	11549.48*** (9254.48)***
Rep/location	7	702.17 (474.69)
Line	47	4964.87*** (3272.67)***
Pathotype × line	141	1398.21*** (956.30)***
Error 1	329	306.91 (225.68)
Time	1	3065.10*** (3291.74)***
Pathotype × time	3	428.04*** (270.02)***
Line × time	47	92.67*** (63.28)***
Pathotype × line × time	141	37.06** (27.80)**
Error 2	336	25.55 (20.02)

[a] Values in parentheses are based on arcsine-transformed data. ** and *** = significant at $P < 0.01$ and $P < 0.001$, respectively.

types of *S. graminicola* has provided lines with resistance to multiple pathotypes both under multilocation field and greenhouse environments. Genetic diversity for downy mildew resistance in A-lines was evident from variable incidence levels to four pathotypes, both in field nurseries and in greenhouse tests. Disease incidence in A-lines was quite variable, but the incidence levels on the susceptible control 7042S indicated high and adequate disease pressure in all tests. There was also variation in disease incidence between field nurseries at different locations, and those between field and greenhouse tests against a particular pathotype. In addition to genetic differences in host lines, there are several weather factors that influence downy mildew infection and symptom expression under field conditions (6,11,15,16). For instance, increase in disease incidence from 30- to 60-day recording in 20 of the 48 lines in the disease nursery at Mysore could be attributed to the prevalence of more conducive environments at a later part of plant growth than at the seedling stage. Since downy mildew infection in pearl millet is systemic, oftentimes symptoms and sporulation can be suppressed under unfavorable weather conditions.

The difference in results between field and greenhouse evaluations against the same pathotype isolates could be attributed to several variable factors operating in the fields. Environments and inoculum are more variable in field nurseries than in the greenhouse. In the field, infection to seedlings occurs both by soilborne oospores (primary infection) and airborne zoospores (secondary infection from infector rows), while in the greenhouse it is only due to zoospores. The fact that field resistance of

Table 3. Downy mildew reactions of pearl millet male-sterile lines (A-lines) against four pathotypes of *Sclerospora graminicola* under field (FD) and greenhouse (GH) conditions

	Downy mildew incidence (%)							
	Patancheru		Mysore		Durgapura		Jalna	
Designation	FD[a]	GH[b]	FD	GH	FD	GH	FD	GH
863A	0	0	8	2	0	0	0	0
ICMA 87001	2	4	12	1	26	11	2	57
ICMA 88004	0	0	6	1	1	3	0	0
ICMA 88006	9	0	7	1	8	7	11	7
ICMA 89111	4	6	9	5	43	26	6	15
ICMA 91222	7	0	17	6	1	24	29	77
ICMA 91444	10	3	32	6	25	4	44	39
ICMA 91666	3	0	8	4	58	2	13	52
ICMA 91777	7	2	13	2	16	5	31	83
ICMA 92111	0	0	6	6	0	9	3	25
ICMA 92333	8	59	48	12	8	28	23	32
ICMA 92444	12	26	21	20	1	29	5	15
ICMA 92666	3	77	14	61	9	66	25	57
ICMA 92777	0	4	7	3	3	0	4	0
ICMA 92888	2	34	59	28	0	19	3	50
ICMA 93111	3	0	11	4	4	5	23	14
ICMA 93222	4	44	30	29	1	20	63	49
ICMA 93333	0	30	10	10	0	15	35	10
ICMA 94111	4	1	9	13	44	17	10	28
ICMA 94222	8	0	14	15	0	5	13	87
ICMA 94333	4	1	10	0	2	1	0	0
ICMA 94444	0	0	4	7	68	4	0	16
ICMA 94555	8	0	9	1	20	6	9	40
ICMA 95111	5	0	11	1	3	2	4	33
ICMA 95222	4	0	8	2	1	4	59	66
ICMA 95333	8	43	10	46	1	34	76	39
ICMA 95444	0	4	10	5	0	6	76	6
ICMA 95555	18	1	79	2	7	<1	38	3
ICMA 96111	8	10	9	13	4	6	56	22
ICMA 96222	0	1	14	12	2	2	3	35
ICMA 96333	3	0	16	0	0	3	0	11
ICMA 96444	2	2	10	10	8	2	47	79
ICMA 96555	7	0	9	7	16	5	15	72
ICMA 96666	0	6	10	3	0	2	3	7
ICMA 97111	5	88	10	19	3	20	9	10
ICMA 97222	25	44	12	23	5	33	17	10
ICMA 97333	3	0	8	4	12	3	7	18
ICMA 97444	5	0	55	1	6	0	0	3
ICMA 97555	4	0	7	1	7	<1	24	10
ICMA 98111	7	0	8	1	1	<1	4	0
ICMA 98222	4	0	10	0	1	<1	0	0
ICMA 98666	20	0	55	12	0	11	3	19
Controls								
81A	2	25	10	33	14	32	61	17
841 A	5	0	8	2	0	9	3	4
843A	66	94	82	45	39	24	46	43
852A	13	0	79	18	8	4	24	54
MBH110 (hybrid)	0	0	10	<1	13	<1	81	81
7042S (line)	100	95	93	91	96	91	84	70
SE	±5.7	±1.6	±6.9	±1.8	±7.4	±2.1	±8.4	±2.6
CV (%)	13.3	2.0	20.1	6.3	3.0	3.2	11.2	2.8

[a] Mean of three replications, except Patancheru (two replications) with two rows per plot per replication and 100 to 120 plants per plot.
[b] Mean of two runs with three replications per run and 60 to 80 seedlings per replication per line.

eight of the nine tested A-lines was confirmed in two greenhouse tests indicates the value, effectiveness, and usefulness of the greenhouse evaluation.

Greenhouse evaluation of A-lines against the four pathotypes provided comparatively more uniform results (with low coefficient of variations) in the two runs than did the field evaluation. Among the three sources of variation (line, pathotype, and line × pathotype), the largest proportion of variability for downy mildew incidence was accounted for by lines, followed by line × pathotype interaction and pathotypes, both in field and greenhouse tests (Tables 2 and 4). The largest number of A-lines (38 out of 42) among the test entries was resistant in the field nursery at Patancheru and the lowest number (22) at Jalna, with Durgapura and Mysore falling between the two, in that order. This difference in the number of resistant lines among the locations might arise either from the environmental differences or from differences among the dominant genotypes in the pathogen populations, or from a combination of both factors.

Greenhouse evaluation of these A-lines with pathotypes from the same four locations also showed a similar pattern, with the largest number of test A-lines (33) being resistant to Patancheru pathotype and the lowest number (15) being resistant to Jalna pathotype, confirming that Jalna pathotype is more virulent than Durgapura and Mysore pathotypes. However, it cannot be claimed that these three pathotypes are necessarily more virulent than the Patancheru pathotype. The largest number of A-lines resistant to Patancheru pathotype may simply be for the reason that these A-lines have been bred for their resistance using the Patancheru disease nursery over several seasons. Earlier studies (13,23) have shown that the four pathotypes used in this study are quite diverse for their virulence and genetic makeup, and that these are present in the major pearl millet growing areas of India. Yet more than 40% of the A-lines bred for resistance at Patancheru were found to be resistant to a pathotype from another location. This could result from chance fixation of genes responsible for resistance against pathotypes from other locations; or a Patancheru pathotype may consist of a low frequency of those pathogen genotypes dominant at other locations, permitting simultaneous selection for resistance to multiple pathotypes. Results of a serial passage study with Patancheru pathotype on several differential host genotypes showed that this pathogen population was a heterogeneous mixture of several genotypes that evolved during serial passage into divergent subpopulations with differential pathogenic reaction (24).

Inbred line 843B is maintainer of 843A, which is the best source of earliness combined with large seed size in a dwarf and agronomically elite background (18). Thus, in A-line breeding at ICRISAT, Patancheru, extensive use of 843B has been made in crosses, with the result that it is one of the parents in almost all the A-lines included in this study. However, 843B is highly susceptible to downy mildew in all the pearl millet growing areas of India. Clearly, then, resistance in the test A-lines came from the other parents used in crosses, almost all of which are of western African origin. It is well known that pearl millet lines that are resistant in western and central Africa (WCA) are mostly resistant in India, but not vice versa (4,5,15,19). Similarly, resistance in

Table 4. Analysis of variance for downy mildew incidence of 48 pearl millet lines against four pathotypes in two runs in a greenhouse experiment

Source of variation	df	Mean square[a]
Replication	2	5.79 (5.03)
Run	1	0.04 (0.41)
Pathotype	3	25958.20*** (19437.62)***
Line	47	6273.20*** (4314.52)***
Run × pathotype	3	79.51 (97.40)
Run × line	47	8.99 (14.62)
Pathotype × line	141	1547.88*** (935.84)***
Run × pathotype × line	141	8.46 (16.57)
Error	766	25.65 (23.04)

[a] Values inside parentheses are for arcsine-transformed data. *** = significant at $P < 0.001$.

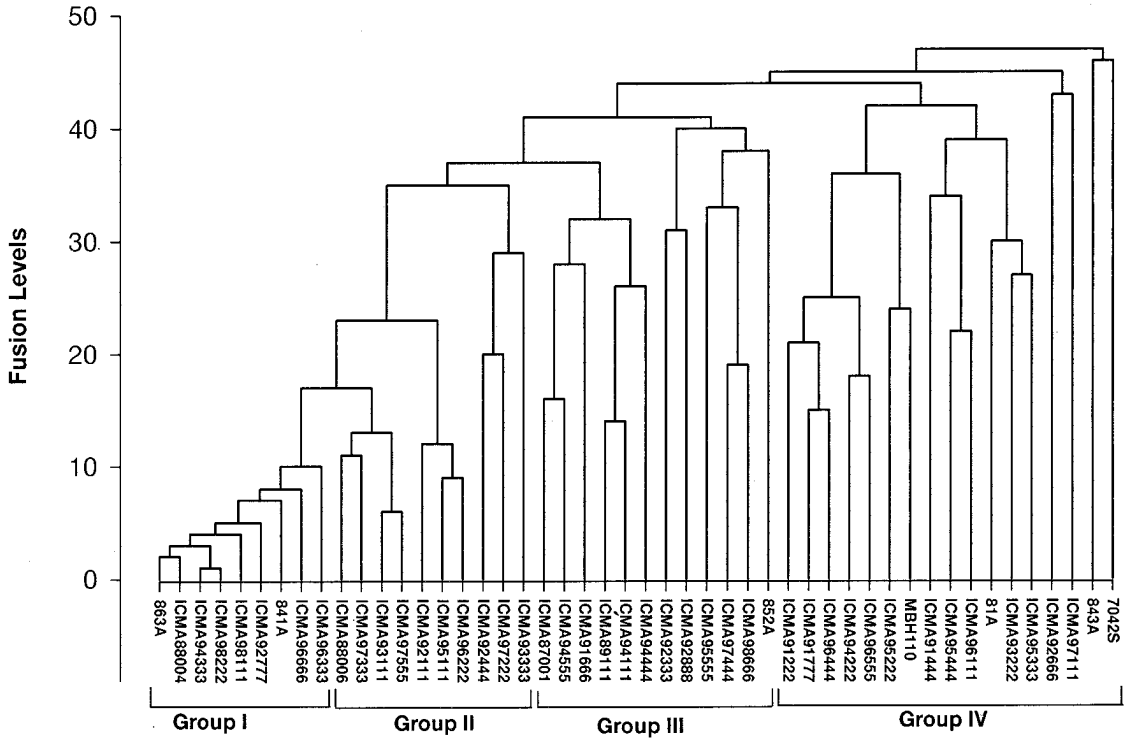

Fig. 1. Classification of 48 pearl millet lines based on average linkage cluster analysis using Euclidian distance of downy mildew incidence (%) caused by *Sclerospora graminicola* in disease nurseries at four locations and four pathotypes in greenhouse experiments: group I = resistant, group II = moderately resistant, group III = susceptible, and group IV = highly susceptible.

line ICMA 88004 seems to have come from the parental line from Togo 11-5 that has shown resistance to downy mildew in field tests at Patancheru (17), and resistance in ICMA 92777 is from either or both of the smut and downy mildew resistant parental lines ICMPS 500-4-4-3 and ICMPS 1800, which have been derived from crosses involving lines from WCA (20). Identification of eight A-lines (863A, ICMA 88004, -92777, -94333, -96666, -98111, -98222, and 841A), which were found to be resistant to all four pathotypes in both field and greenhouse evaluations, provides opportunity for their direct use in breeding hybrid cultivars with resistance to multiple pathotypes of *S. graminicola*. Use of a resistant male parent that can combine well with these A-lines for high grain yield and increased downy mildew resistance would provide durability and wider adaptability of hybrid cultivars in India. These lines also provide a genetic resource in improved genetic background for their further utilization in a crossing program for breeding new A-lines with stable downy mildew resistance. Further, the maintainer counterpart of these A-lines can also be used to develop a downy mildew resistant maintainer composite for eventual use in breeding new A-lines with stable downy mildew resistance. However, since three of these test A-lines are direct derivatives from *Iniadi* germ plasm and two other A-lines also involve *Iniadi* germ plasm in their parentage, such a composite will have a narrow genetic base for agronomic traits, although high resistance to various pathotypes of downy mildew. Therefore, a better strategy would be to include maintainers of 10 A-lines from group II (moderately resistant) to develop a composite with a broader genetic base for agronomic traits.

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