Evidence for a New Virulent Pathotype of *Sclerospora graminicola* on Pearl Millet

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Abstract

Field surveys of pearl millet (*Pennisetum glaucum*) crops in Maharashtra, India during the past four years (1993–96) indicated high incidence of downy mildew (*Sclerospora graminicola*) on several new F_1 hybrid cultivars. Disease incidence varied considerably within and across fields of several cultivars. Some of the more popular private sector hybrids (MLBH 104, MLBH 267, BK 560, Eknath 201, JKBH 26, Pioneer 7602) recorded downy mildew incidence up to 80–100% in some years in certain fields. In contrast to F_1 hybrids, open–pollinated cultivars Mallikarjuna and ICTP 8203 recorded trace or no downy mildew incidence. In a greenhouse experiment, *S. graminicola* isolates Sg 008 (from NHB 3), Sg 010 (MBH 110), Sg 021 (MLBH 104), Sg 024 (BK 560), and Sg 026 (Nath 4209) showed differential virulence on a set of host differential lines (NHB 3, 5141A, MBH 110, BK 560, and 852B). DNA fingerprinting of isolates, using a microsatellite $(GATA)_4$ revealed high levels of polymorphism among the isolates. Both virulence and DNA fingerprinting analyses showed that isolate Sg 021 from a popular hybrid MLBH 104 was quite distinct from those from MBH 110, BK 560 and others. The cluster analyses of virulence data and DNA fingerprinting data classified the isolates into four groups, although there was no complete agreement between the two groupings. The results indicate the evolution of a new virulent pathotype (Sg 021) specific to a widely grown hybrid MLBH 104, which has caused substantial damage to the crop in Maharashtra during the past three years. A strategy to monitor and manage downy mildew in pearl millet is discussed.

महाराष्ट्र राज्य में विगत चार वर्षों (1993-96) के दौरान किए गए बाजरे के सर्वेक्षणों में मृदुरोमिल *(स्क्लेरोस्पोरा ग्रेमीनीकोला)* का F_1 संकर कृषिजोपजाति पर अधिकतम आपतन दिखाई दिया। खेत में और खेत के पार कई कृषि जोपजातियों पर रोग आपतन पर्याप्त रूप से भिन्न था। कुछ खेतों में तो किसी किसी वर्ष निजी सेक्टर के अधिक प्रचलित संकर (MLBH 104, MLBH 267, BK 560, एकनाथ 201, JKBH 26, पायोनीयर 7602) पर रोग आपतन 80-100% तक अंकित किया गया। F_1 संकर के विपरीत प्रकृति में परागित कृषिजोपजाति मल्लिकार्जुन और ICTP 8203 या तो रोग रहित थी या अंश मात्र मृदुरोमिल आपतन था। (ग्रीन हाउस) हरित गृह में किए गए प्रयोगों में *एस. ग्रेमिनिकोला* पृथक्कृत Sg 008 (NHB 3 से), Sg 010 (MBH 110 से), Sg 021 (MLBH 104 से), Sg 624 (BR 560 से) और Sg 026 (नाथ 4209 से) ने परपोषी विभेदकों (NHB 3, 5141 A, MBH-110, BK 560 और 852 B) पर विभेद उग्रता दिखाई। पृथक्कृत को माइक्रोसेटेलाइट (GATA) का उपयोग करते हुए डी एन ए फिंगर प्रिंटिंग करने से पृथक्कृतों में उच्च स्तरीय बहुरूपता ज्ञात हुई। उग्रता और डी.एन.ए. फिंगर प्रिंटिंग के विश्लेषणों ने यह दिखाया कि प्रचलित संकरMBH-104 से प्राप्त पृथक्कृत Sg 021, अन्य MBH-110, BK 560 संकर MLBH-104 से पूर्ण रूप से भिन्न था। एक साथ उग्रता विश्लेषण आँकड़े और डी.एन.ए. फिंगर प्रिंटिंग आँकड़े के

पृथक्कृतों को चार समूहों में वर्गीकृत कर दिया, यद्यपि यह देखा गया कि किसी भी दो समूहों में पूर्ण समानता नहीं थी। उपरोक्त परिणाम एक नए उग्र पेथोटाइप (Sg 021), जो कि अधिकतर क्षेत्र में उगाई जाने वाली संकर MLBH-104 पर विशेष तौर से आता है और जिसने महाराष्ट्र में विगत तीन वर्षों में इस फसल को अत्यधिक क्षति पहुँचाई है, का विकास होना बताता है। बाजरे में मृदुरोमिल को मोनिटर और प्रबन्धन करने की युक्ति का विवेचन किया गया है।

Variation in plant pathogenic fungi arises largely through sexual processes, heterozygosity and somatic recombination, mutation, and selection. Large shifts in virulence occurs due to changes in host cultivar and environment. Virulence has been used as the genetic marker in studies where variability has been assessed through virulence surveys, using host differentials containing different resistance genes (Wolfe and Knott, 1982). *Sclerospora graminicola* (Sacc.) Schroet., the causal agent of downy mildew in pearl millet (*Pennisetum glaucum* (L.) R. Br.), is an obligate biotroph, which reproduces asexually by means of sporangia that liberate motile zoospores, and sexually through oospores (Williams, 1984). The fungus is largely heterothallic (Idris and Ball, 1984; Michelmore *et al.*, 1982). Host cultivar–directed virulence selection in *S. graminicola* population has been demonstrated through serial passage experiments in a greenhouse (Thakur *et al.*, 1992). These results have further been confirmed by molecular characterization of pearl millet cultivar–specific pathotypes of *S. graminicola*, using mini– and microsatellites (Sastry *et al.*, 1995).

Genetically uniform single–cross hybrids that were grown widely by farmers for several years in the same fields succumbed to downy mildew and these (HB 3, NHB 3, and BJ 104) were withdrawn (Singh *et al.*, 1993). Recent surveys in farmers' fields have shown increased incidence of downy mildew on several new pearl millet hybrids in Maharashtra over a period of the past few years. A highly popular hybrid MBH 110 succumbed to downy mildew in Maharashtra few years ago and subsequently it was withdrawn from cultivation (Thakur *et al.*, 1992; Hash, 1997). Virulence studies of isolates from MBH 110 indicated the emergence of new pathotype specific to MBH 110, and that it was different from those reported on NHB 3 and BJ 104 (Thakur *et al.*, 1992). In this paper we report the results of field surveys and studies confirming the emergence of a new virulent pathotype specific to MLBH 104, another commercial hybrid highly popular in Maharshtra.

Materials and Methods

Field survey. In collaboration with the All India Coordinated Pearl Millet Improvement Project (AICPMIP) scientists, pearl millet crops were surveyed during the rainy season 1993–96 in farmers' fields in Maharashtra, India for recording downy mildew incidence (Table 1). A roving survey was planned in each crop season to examine the crop at the tillering to flowering stages. Pearl millet fields, nearest to the road were observed, and in each field five random microplots (2 rows x 5m) with at least 50 plants/microplot were examined for

Table 1. Pearl millet cultivars seen during downy mildew survey in Maharashtra during 1993–96

Cultivar	Seed company/organization
MLBH 104	Mahendra Hybrid Seeds Co., Ltd. Jalna, Maharashtra (MS)
MLBH 267	Mahendra Hybrid Seeds Co., Ltd. Jalna, MS
MLBH 287	Mahendra Hybrid Seeds Co., Ltd. Jalna, MS
Nath 4209	Nath Seeds Limited, Aurangabad, MS
Eknath 201	Nath Seeds Limited, Aurangabad, MS
GK 1004	Ganga Agricultural Seeds Ltd., Hyderabad, Andhra Pradesh (AP)
GK 1006	Ganga Agricultural Seeds Ltd., Hyderabad, AP
Proagro 7701	Proagro Seeds Ltd., Hyderabad, AP
JKBH 26	JK Agricultural Genetics, Hyderabad, AP
Pioneer 7602	SPIC PHI Seeds Ltd., Aurangabad, AP
BK 560	All India Coordinated Pearl Millet Improvement Project (AICPMIP)
Shradda	Mahatma Phule Krishi Vidyapeeth, Rahuri, MS
MH 179	ICRISAT, Patancheru, AP
ICTP 8203	ICRISAT, Patancheru, AP
Mallikarjuna	Andhra Pradesh Agricultural University, Palem, AP

downy mildew symptoms. Counts were taken for the total and diseased plants in each microplot and the mean downy mildew incidence for each field was calculated. Data were also collected for the cultivar designation, the seed company which has released the cultivar. Downy mildew samples from each cultivar were collected for obtaining oospores which were used to generate sporangial inocula for pathogenicity test at ICRISAT, Patancheru.

Pathogen isolates. Downy mildew infected leaf samples from the cultivars in farmers' fields were collected to obtain oospore inoculum. Sporangial inoculum from the oospore inoculum were generated by infesting the seed of a highly susceptible pearl millet line 7042S and growing the seedlings in pots. The isolates thus obtained, were maintained on 7042S were designated as: Sg 008 (from NHB 3), Sg 010 (MBH 110), Sg 021 (MLBH 104), Sg 022 (Mallikarjuna), Sg 024 (BK 560), Sg 026 (Nath 4209), and Sg 028 (Kaudal local) and were used for inoculation. Each isolate was kept in polyacrylic isolation chamber in a greenhouse at 25°C. Sporangial inoculum was increased by inoculating pot–grown seedlings of 7042S, using the standard inoculation method (Singh *et al.*, 1993).

Host lines. Pearl millet lines/cultivars NHB 3, 5141B, MBH 110, BK 560 and 852B were used as host differentials, based on their earlier reactions in International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN) (Thakur, 1995). Seeds were surface sterilized with 0.1% $HgCl_2$ for 4–5 min and dried on blotting paper in the laboratory before sowing in pots (15cm dia) filled with autoclaved mix of soil, sand and farm–yard manure (3:2:2 by vol.).

Inoculation. Seedlings at the 2–leaf stage were spray–inoculated with sporangial suspension (1 x 10^5 sporangia ml^{-1}), incubated at 20°C and >95% RH for 16h and then transferred to 25°C in a greenhouse (Singh *et al.*, 1993). The experiment was conducted in a completely randomized design with four replications. Each replication had at least 100 seedlings in two pots. The experiment was repeated once.

Data collection and analysis: Counts were taken for numbers of total and diseased plants 14 days after inoculation and percentage disease incidence was calculated. Data from each experimental run were subjected to analysis of variance using GENSTAT (Rothamested Experiment Station, Herpenden, Herts, UK) to compare the differences among isolates for their disease inducing capacity. A cluster analysis, using the average linkage method, of mean downy mildew incidence data was done to classify the isolates into different virulence groups. A dendrogram was prepared to show the similarities among the isolates.

DNA isolation, restriction enzyme digestion and gel electrophoresis. Sporangia were harvested from sporulating infected leaves of pearl millet seedlings inoculated with six isolates separately in ice–cold sterile distilled water and pelleted in Sorvall RC centrifuge at 4000g for 20 min at 5°C. High molecular weight DNA was isolated from the sporangial pellet as described by Sastry *et al.* (1995).

About 8–10 ug of DNA was digested with a restriction enzyme *MspI* (according to supplier's instructions) and electrophoresed on 1.2% agrose gel in TPE buffer (90mM Tris–phosphate, 2mM EDTA, pH 7.5). The gels were stained in ethidium bromide, photographed and dried in a gel dryer.

Southern hybridization. An oligonucleotide probe $(GATA)_4$ was 5'–end–labeled by T4 polynucleotide kinase as described by Sambrook *et al.* (1989). Dry gels were denatured, neutralized and hybridized at 35° C and given stringent washing according to Schafer *et al.* (1988). The blots/dry gels were exposed to x–ray films (Kodok, USA) for a specific period depending on the intensity of hybridization signal.

Data recording and analysis. The relatedness of the six isolates was estimated by means of scorable bands. Differences in banding pattern were scored on the basis of absence or presence of a band. Similarity indices expressing the probability that a fragment in one isolate is also found in another for all pairwise comparisons were made (Wetton *et al.*, 1987). Cluster analysis of data was done using the statistical software package SYSTAT 5.1. A dendrogram showing the mean similarities between groups of different isolates was generated.

Results

Field survey. Downy mildew incidence data from field surveys indicated a general trend in increasing susceptibility of commercial hybrids (Table 2). Hybrid MLBH 104 that has already been in commercial production in Maharashtra for the past 4 years showed between 80 and 90% incidence in some fields in 1995 and 1996. Similarly, another hybrid MLBH 267 which had 0 to 40% disease during 1993–95, showed up to 90% disease in 1996. Some of the recently commercialized hybrids, such as Eknath 201, GK 1006, JKBH 26 and Pioneer 7602 exhibited up to 75% or more disease in certain fields in 1996. In contrast to single–cross hybrids, the open–pollinated varieties ICTP 8203 and Mallikarjuna showed either no disease or very little disease. There were some other hybrids, such as Shradda and MH 179 showed no disease as these were probably grown for the first time in the fields visited.

Pathogenicity test. All seven isolates were virulent on NHB 3, 5141B, and BK 560 in both experimental runs although they induced different levels of disease incidence (Table 3). Isolates Sg 008 and Sg 010 did not produce symptoms on 852B and thus was avirulent, while all except two (Sg 010 and Sg 028) were avirulent on MBH 110. Isolate Sg 008

Table 2. Downy mildew incidence on various pearl millet cultivars in farmers' fields in Maharashtra, during surveys 1993–96

	Downy mildew incidence (%)[a]			
Cultivar[b]	1993	1994	1995	1996
MLBH 104	0–53	10–20	5–90	10–80
MLBH 267	0	10–40	2–20	1–90
MLBH 287	–[c]	–	–	20–50
Nath 4209	27–53	–	–	–
Eknath 201	–	–	5–15	20–90
GK 1004	–	–	30–80	1–20
GK 1006	–	–	0–2	20–75
Proagro 7701	–	–	20–40	0–1
JKBH 26	–	–	–	50–80
Pioneer 7602	–	–	–	90–100
BK 560	2–90	20–70	–	–
Shradda	–	–	–	0
MH 179	–	–	–	0
ICTP 8203	0	0	0–2	0
Mallikarjuna	0	–	0	0

[a]Based on five samples of 50 plants each in a field, and number of fields varied from 1 to 60 for each cultivar.
[b]All cultivars are F_1 hybrids, except ICTP 8203 and Mallikarjuna which are open–pollinated varieties.
[c] Not encountered during the survey.

Table 3. Downy mildew incidence (%)[a] on five pearl millet lines induced by seven isolates of *Sclerospora graminicola* from different cultivars in two runs

	NHB 3		5141 B		MBH 110		BK 560		852 B	
Isolate (source)	I	II	I	II	I	II	I	II	I	II
Sg 008 (NHB 3)	88	91	88	89	0	0	63	63	0	0
Sg 010 (MBH 110)	89	93	85	87	59	57	47	51	0	0
Sg 021 (MLBH 104)	87	96	39	54	0	0	27	27	27	34
Sg 022 (Mallikarjuna)	49	40	91	87	0	0	27	23	30	35
Sg 024 (BK 560)	94	89	72	82	0	0	52	49	18	21
Sg 026 (Nath 4209)	89	87	58	64	0	0	39	39	81	69
Sg 028 (Kaudal local)	65	53	17	12	5	3	23	19	30	38

LSD ($P < 0.01$): isolate x host line interaction: run I = 7.00; run II = 7.50.
[a]Mean of 4 replications with at least 100 seedlings in each replication.

from NHB 3 induced high disease on its own host NHB 3, 5141B (line related to NHB 3), and BK 560 (again closely related to NHB 3), but not on MBH 110 and 852 B whioh are widely unrelated to NHB 3. Similarly, isolate Sg 010 from MBH 110 induced disease on its own host and other lines, except 852B. Isolate Sg 021 from MLBH 104 did not cause disease on MBH 110, but caused on 852B. Isolate Sg 028 from Kaudal local cultivar induced disease on all lines though the incidence was much lower than those caused by other isolates (Table 3). Analysis of variance indicated highly significant ($P<.001$) isolate x host line interaction suggesting the occurrence of host–pathogen specificity for virulence (Table 4). The hierarchieal cluster analysis of mean downy mildew incidence data across five host lines classified the seven isolates into four groups (Fig. 1). Isolate Sg 021 showed closeness to Sg 022, but was quite different from the other isolates Sg 008, Sg 010 and Sg 024.

Table 4. Analysis of variance for downy mildew incidence in two experimental runs in a greenhouse

		Mean sum of square	
Source of variation	df	Run I	Run II
Isolates (I)	6	1994.84***	2332.41***
Host lines (H)	4	22764.40***	22938.98***
I x H interaction	24	2012.69***	2008.80***
Error	105	14.20	16.14

***Significant at $P < 0.001$

DNA fingerprinting. The autoradiogram of DNA fingerprinting (Fig. 2) showed variable polymor-

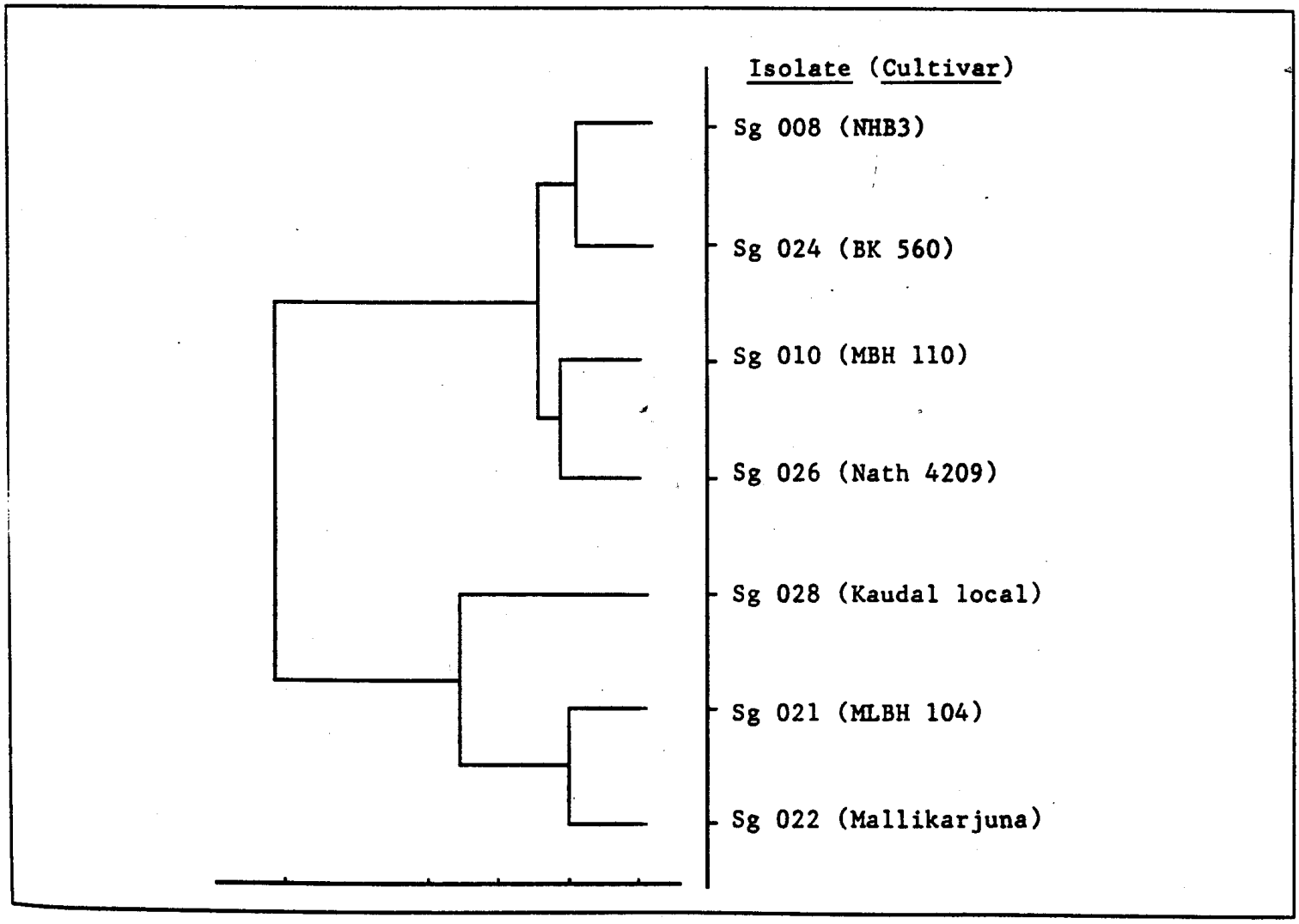

Figure 1. Dendrogram of seven isolates of *Sclerospora graminicola*, based on cluster analysis of mean downy mildew incidence recorded on five pearl millet lines in two experimental runs in a greenhouse. Note that the isolate Sg 021 from MLBH 104 lies in a distinct cluster than Sg 010 from MBH 110.

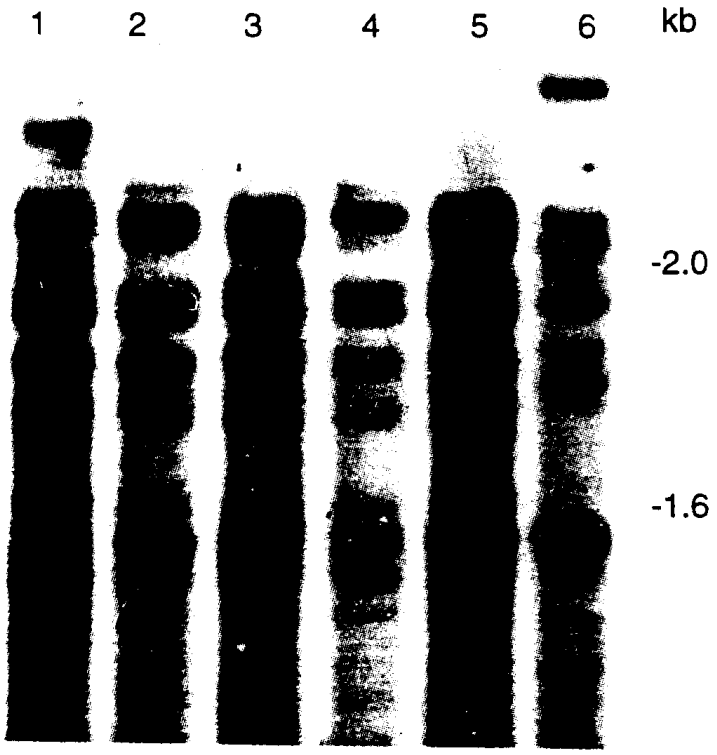

Figure 2. Autoradiogram of the DNA fingerprints of six isolates of *Sclerospora graminicola*. *Fungal DNA digested* with restriction enzyme *Msp*I and hybridized to the microsatellite probe (GATA)$_4$. Lane 1: Sg 024 (from BK 560); lane 2: Sg 026 (Nath 4209); lane 3: Sg 028 (Kaudal local); lane 4: Sg 022 (Mallikarjuna); lane 5: Sg 021 (MLBH 104); and lane 6: Sg 010 (MBH 110). Molecular weight based on the standards (kb) is indicated in the right margin.

phism among the isolates. The banding pattern for the isolate Sg 010 (MBH 110) was distinct from Sg 021 (MLBH 104) and those of other isolates. Similarly, banding in isolate Sg 024 (BK 560) differed from other isolates in one distinct band. Isolate Sg 021 had different banding pattern from those of others. The cluster analysis of band scores (Fig. 3) also revealed four distinct grouping of isolates. Isolates Sg 026 (Nath 4209) and Sg 028 (Kaudal local) grouped together while Sg 022 (Mallikarjuna) and Sg 021 formed another group.

Discussion

Both pearl millet and *S. graminicola* are highly out crossing, and thus genetically highly variable. It is, therefore, unlikely that genes for resistance in the natural population of host and genes for virulence in the pathogen will be homozygous and stable. In India, commercial cultivation of genetically uniform pearl millet single–cross hybrid cultivars BJ 104 and MBH 110 during the late 1970s and early 1980s provided strong selection pressure on the pathogen population for a shift to host-specific virulences. We demonstrated through a controlled inoculation experiment the response of host cultivar–directed selection for virulence in a *S. graminicola* field population from ICRISAT, Patancheru (Thakur *et al.*, 1992).

There are circumstantial evidences of evolution of host cultivar–specific virulences in *S. graminicola* in India due to commercial cultivation of single–cross hybrids since the early 1970s. Hybrid cultivars, HB 3, NHB 3, BJ 104, and MBH 110 succumbed to downy mildew within 4–7 years of their cultivation during 1970–1988 (Singh *et al.*, 1993; Thakur *et al.*, 1995; Hash, 1997). Physiologic specialization in *S. graminicola* (Bhat, 1973; Shetty and Ahmed, 1981), and variation in the pathogen populations were demonstrated among isolates from India and western Africa (Ball *et al.*, 1986; Singh and Singh, 1987). During the last 10 years private sector seed industries have taken up the front seat in production and release of commercial hybrids. Our survey data from the state of Maharashtra for the past four years indicate the increasing trend of introduction of new hybrids by private seed companies. Some of the high yielding hybrids reach farmers' fields faster than others and become highly popular. Chopra *et al.* (1997) commented that MLBH 104 would gradually be withdrawn in favour of new hybrids MLBH 267, MLBH 285, and MLBH 287, but even the new hybrids MLBH 267 recorded up to 90% and MLBH 287 up to 50% downy mildew incidence in certain fields during the 1996 field survey (Table 2). These genetically uniform single–cross hybrids provide tremendous selection pressure on the pathogen populations which leads to emergence of a new virulent pathotype within 2–3 years, and subsequently the hybrids become susceptible. In contrast to single–cross hybrids, open pollinated varieties, ICTP 8203 which is very widely grown in Maharashtra, exhibited only trace of downy mildew symptoms. This reinforces the hypothesis that genetic heterogeneity in a cultivar that offers certain level of resistance to infection by the pathogen,

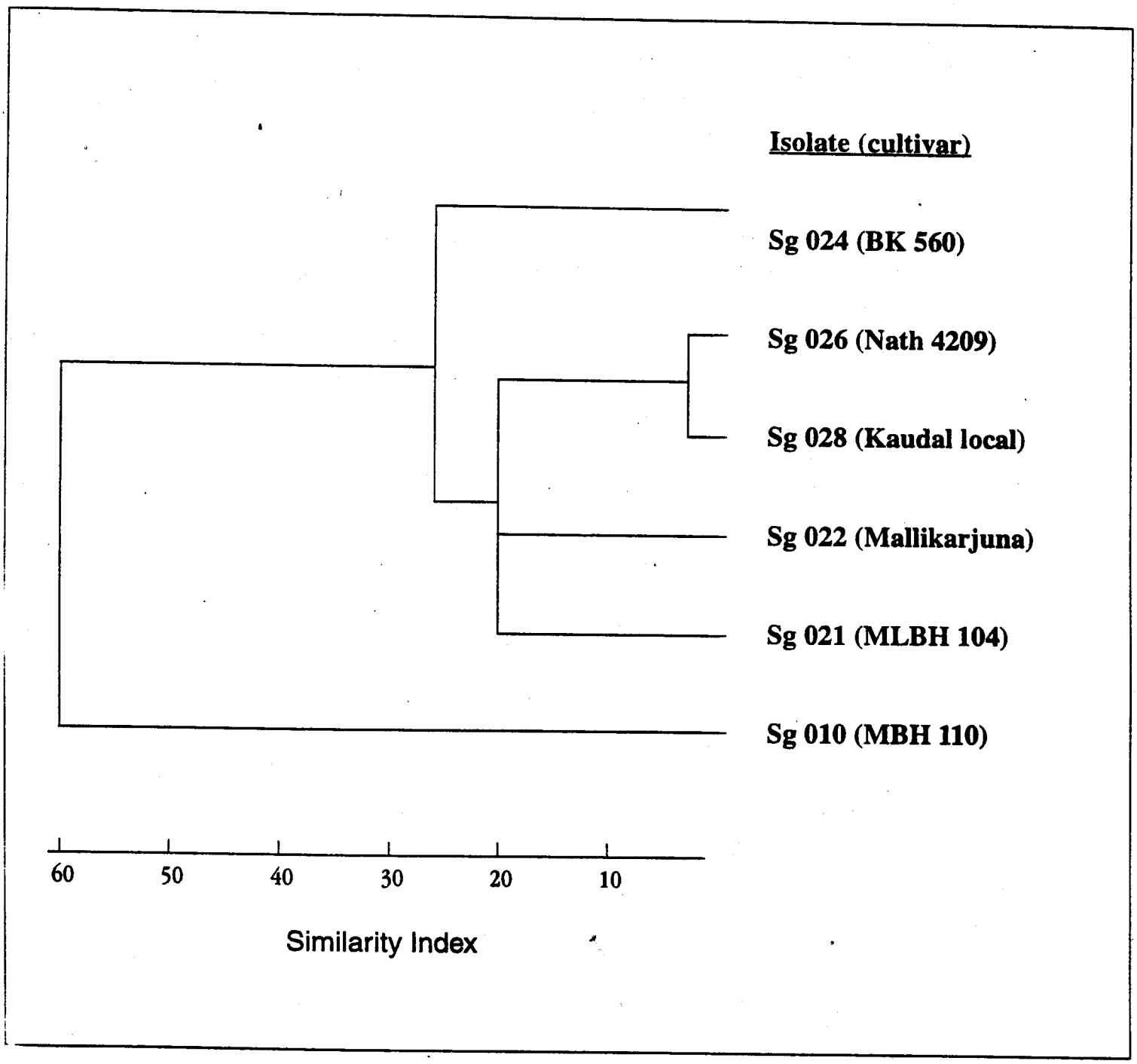

Figure 3. Dendrogram of six isolates of *Sclerospora graminicola* with microsatellite probe $(GATA)_4$: Cluster analysis of data obtained from the hybridization of *Msp*I–digested DNA with $(GATA)_4$. Note that the isolate Sg 021 from MLBH 104 is quite distinct from that of Sg 010 from MBH 110 as depicted in Fig. 1.

prevents host–specific selection to operate in the pathogen population for virulence.

Our virulence data indicate the emergence of a new pathotype specific to hybrid MLBH 104 which has been popular in Maharashtra after the withdrawal of MBH 110 a few years ago. The fact that isolate Sg 021 does not infect MBH 110, but infects other host lines, strongly supports the view of host cultivar–directed virulence selection (Leonard, 1977; Thakur *et al.*, 1992; Thompson and Burdon, 1992; Wolfe and Knott, 1982).

The DNA fingerprinting data of isolates (Fig. 2) demonstrate the genetic differences among the isolates, and the distinct banding pattern for Sg 021 from the others. Sastry *et al.* (1995) used this technique successfully to detect polymorphism in host cultivar–specific populations of *S. graminicola*. The dendrograms of virulence data largely

agree with that of DNA fingerprinting data on the grouping of different isolates. Both results clearly distinguish isolate Sg 021 (MLBH 104) from those of Sg 010 (MBH 110) and Sg 024 (BK 560).

The host–pathogen interaction in the pearl millet–*S. graminicola* system is highly complex, because of high levels of genetic heterogeneity both in the host and the pathogen. Resistance to downy mildew has been demonstrated to be highly pathogen population specific, and several quantitative trait loci (QTLs) for downy mildew resistance have recently been identified in pearl millet (Jones *et al.*, 1995). Evolution of new virulence in *S. graminicola* populations, like in other pathosystems, is a continuous process, often triggered by the release of new host cultivars. The strategy to manage this disease will therefore, involve: constant monitoring of pearl millet cultivars in farmers' fields for resistance breakdown and evolution of new virulence; identification of new sources of resistance to the new virulent pathotypes; resistance gene pyramiding; and deployment of newer resistant cultivars.

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References

Ball, S.L., D.J. Pike and C.Y.Burridge. 1986. Characterization of populations of *Sclerospora graminicola. Ann. Appl. Biol.* 108:519–526.

Bhat, S.S. 1973. *Investigations on the biology and control of Sclerospora graminicola on bajra.* Ph.D. Thesis. Dept. of Post Graduate Studies and Research in Botany. Univ. of Mysore, Mysore, India. 165 pp.

Chopra, K.R., K.K. Thimmaiah and P.N. Gelda. 1997. Pearl millet hybrid MLBH 104 in Maharashtra. In Bantilan, M.C.S. and Joshi, P.K. (eds.). *Integrating Research Evaluation Efforts: Proceedings of an International Workshop.* 14–16 dec 1994. ICRISAT, Patancheru, India. Patancheru 502 324, Andhra Pradesh, india: International Crops Research Institute for the semi–Arid Tropics. pp 133–135.

Idris, M.O. and S.L. Ball. 1984. Inter– and intracontinental sexual compatibility in *Sclerospora graminicola. Plant Pathol.* 33:219–223.

Jones, E.S., C.J. Liu, M.D. Gale, C.T. Hash and J.R. Witcombe. 1995. Mapping quantitative trait loci for downy mildew resistance in pearl millet. *Theor. Appl. Genet.*91:448–456.

Hash, C.T. 1997. Research on downy mildew of pearl millet. In Bantilan, M.C.S. and Joshi, P.K. (eds.). *Integrating Research Evaluation Efforts: Proceedings of an International Workshop.* 14–16 dec 1994. ICRISAT, Patancheru, India. Patancheru 502 324, Andhra Pradesh, india: International Crops Research Institute for the semi–Arid Tropics. pp 121–128.

Leonard, K.J. 1977. Selection pressures and plant pathogens. *Ann. New York Acad. Sci.* 287:207–222.

Michelmore, R.W., M.N. Pawar and R.J. Williams. 1982. Heterothallism in *Sclerospora graminicola. Phytopathology* 72:1368–1372.

Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. *Cloning : Laboratory manual.* 2nd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Sastry, J.G., W. Ramakrishna, S. Sivaramakrishnan, S., R.P. Thakur, V.S. Gupta, and P.K. Ranjekar. 1995. DNA fingerprinting detects genetic variability in the pearl millet downy mildew pathogen (*Sclerospora graminicola*). *Theor. Appl. Genet.* 91:856–861.

Schafer, R., I.I. Zischler, U. Birsner, A. Becker, and J.T. Epplen. 1988. Optimized oligonucleotide probes for DNA fingerprinting. *Electrophoresis* 9:369–274.

Shetty, H.S. and R. Ahmad. 1981. Physiologic specialization in *Sclerospora graminicola. Indian Phytopath.* 34:307–309.

Singh, S.D. and G. Singh. 1987. Resistance to downy mildew in pearl millet hybrid NHB-3. *Indian Phytopath.*40:178-180.

Singh, S.D., S.B. King and J. Werder. 1993. *Downy mildew disease of pearl millet.* Information Bulletin no. 37. International Crops Research Institute for the Semi–Arid Tropics: Patancheru, A.P. 502 324, India.

Thakur, R.P. 1995. Status of International Sorghum Anthracnose and Pearl Millet Downy Mildew Virulence Nurseries. In: J.F. Leslie and R.A. Frederiksen (eds.), Disease Analysis through Genetics and Biotechnology Interdisciplinary Bridges to Improve Sorghum and Millet Crops. Iowa State University Press, Iowa, Ames. pp 75–92.

Thakur, R.P., K.G. Shetty and S.B. King. 1992. Selection for host-specific virulence in asexual populations of *Sclerospora graminicola*. *Plant Pathol.* 41:626-632.

Thakur, R.P., S. Sivaramakrishnan, J.G. Sastry and P.K. Ranjekar. 1995. Pathogenic and genetic variation in *Sclerospora graminicola*. *The Downy Mildew Fungi, An International Symposium*, Gwatt, Switzerland. 29 Aug–2 Sep, 1995 (Abstr.).

Thompson, J.N. and J.J. Burdon. 1992. Gene–for–gene coevolution between plants and parasites. *Nature* 360:121–125.

Wetton, J.H., R.E. Carter, D.T. Parkin and D. Walters. 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature* 327:147–149.

Williams, R.J. 1984. Downy mildews of tropical cereals. *Advances in Plant Pathology* 2:1-103.

Wolfe, M.S. and D.R. Knott. 1982. Population of plant pathogens: some constraints on analysis of variation in pathogenicity. *Plant Pathol.* 31:79–90.