14. Wang, Z., Mackill, D. J. and Bonman, J. M., Inheritance of partial resistance to blast in *indica* rice cultivars. *Crop Sci.*, 1989, **29**, 848–853.
15. Roumen, E. C., The inheritance of host plant resistance and its effect on the relative infection efficiency of *Magneporthe grisea* in rice cultivars. *Theor. Appl. Genet.*, 1994, **89**, 498–503.
16. Brar, D. S. and Khush, G. S., Alien introgression in rice. *Plant Mol. Biol.*, 1997, **35**, 35–47.
17. Xiao, J., Li, J., Grandillo, S. S., Ahn, N., Yuan, L., Tanskley, S. D. and McCouch, S. R., Identification of trait improving quantitative trait loci alleles from wild rice relative *O. rufipogon*. *Genetics*, 1998, **150**, 988–909.
18. AICRIP Progress Report 2001, vol. 2, p. 3.56 and DRR Screening Nursery 2001, p. 118.
19. Standard Evaluation System for Rice, International Rice Research Institute, Manila, Philippines, 1996.
20. Geddes, A. M. W., Khush, G. S. and Iles, M., The relative importance of crop pests in South Asia. *NRI Bull.*, 1991, 39, p. 102.
21. Sridhar, R., Singh, U. D., Agrawal, P. K. and Reddy, J. N., Usefulness of blast resistance genes and their combinations in different blast endemic locations in India. *IRRN*, 1999, **24**, 22–24.
22. Sharma, T. R., Chauhan, R. S., Singh, B. M., Paul, R., Sagar, V. and Rathour, R., RAPD and pathotype analysis of *Magnaporthe grisea* populations from the northwestern Himalayan region of India. *J. Phytopathol.*, 2002, **150**, 649–656.
23. Padmanabhan, S. Y., Chakrabarti, N. K., Mathur, S. C. and Veeraraghavan, J., Identification of pathogenic races of *Pyricularia oryzae* cav. in India. *Phytopathology*, 1970, **60**, 1574–1577.
24. Bhardwaj, C. L. and Singh, B. M., Pathogenic variation in *Pyricularia oryzae* in Himachal Pradesh. *Indian Phytopathol.*, 1982, **35**, 167–170.
25. Rathour, R., DNA fingerprinting of *Megnaporthe grisea* from rice for virulence diversity. Ph D thesis, HPK Vishvavidyalaya, Palampur, 2000, p. 115.
26. Nottenghem, J. L., Durable resistance to rice blast disease. In *Current Plant Science and Biotechnology in Agriculture* (eds Jacobs, T. H. and Parlevliet, J. E.), Proceedings of International Symposium, Kluwer, Dordrecht, 1993, vol. 18, pp. 125–134.
27. Lee, F. N., Rice breeding programs, blast epidemics and blast management in the United States. In *Rice Blast Disease* (eds Zeigler, R. S., Leong, S. A. and Teng, P. S.), CAB International, UK, 1994, pp. 489–500.
28. Buddehagen, I. W., Disease resistance in rice. In *Durable Resistance in Crop* (ed. Lamberti, F.), Plenum Press, 1983, pp. 401–428.
29. Parlevliet, J. E., Identification and evaluation of quantitative resistance. In *Plant Disease Epidemiology, Genetics, Resistance and Management* (eds Leonard, K. J. and Fry, W. E.), McGraw Hill, New York, 1988, pp. 215–248.
30. Variar, M., Maiti, D., Sinha, P. K., Mandal, N. P. and Shukla, V. D., Recent advances in blast resistance gene deployment in rice. In Proceedings on Boro Rice and Strategies for Enhancing Rice Production and Productivity in the Northeast, DRR, 2004, pp. 112–115.
31. Jeon, J. S., Chen, G. H. Y., Wang, G. L. and Ronald, P. C., Genetic and physical mapping of *Pi5*(t), a locus associated with broad-spectrum resistance to rice blast. *Theor. Appl. Genet.*, 2003, **269**, 280–289.
32. Liu, G., Lu, G., Zing, I. and. Wang, G. L., Two broad-spectrum blast resistance genes, *Pi9*(t) ad *Pi2*(t) are physically linked on rice chromosome. *Mol. Genet. Genom.*, 2002, **267**, 472–480.
33. Muralidharan, K., Krishnaveni, D., Laha, G. S., Reddy, C. S., Srinivasa Prasad, M. and Sridhar, R., Evaluation of blast resistance genes in India. *Rice Genet. Newsl.*, 2003, **20**, 94–96.
34. Inukai, T., Zeimer, R. S., Sarkarung, S., Bronson, M., Dung, L. V., Kinoshita, T. and Nelson, R. J., Development of pre-isogenic lines for rice blast-resistance by marker-aided selection from a recombinant inbred population. *Theor. Appl. Genet.*, 1996, **93**, 560–567.
35. Chen, D. H., Delavina, M., Inukai, T., Mackill, D. J., Ronald, P. C. and Nelson, R. J., Molecular mapping of the blast resistance gene, *Pi44*(t) in a line derived from a durably resistant cultivar. *Theor. Appl. Genet.*, 1999, **98**, 1046–1053.
36. Brar, D. S. and Khush, G. S., Transferring genes from wild species into rice. In *Quantitative Genetics, Genomics and Plant Breeding* (ed. Kang, M. S.), CAB International, 2002, pp. 197–205.

ACKNOWLEDGEMENTS. We thank Dr D. S. Brar, IRRI, Philippines for providing NILs and pyramided lines. We also thank all the scientists responsible for the conduct of the All-India Coordinated Rice Trials (National Screening Nursery and the experiment on field monitoring of virulence of *M. grisea*) at different locations in the country.

Received 14 July 2005; revised accepted 26 October 2006

Characterization of variability among isolates of *Fusarium graminearum* associated with head scab of wheat using DNA markers

M. S. Saharan[1,*], A. Naef[2], J. Kumar[1] and R. Tiwari[1]

[1]Directorate of Wheat Research, Karnal, Haryana 132 001, India
[2]Institute of Plant Sciences, Federal Institute of Technology, Zurich, Switzerland

Head scab of wheat caused by *Fusarium* species is characterized by bleaching of the wheat spike, shrivelled kernels and accumulation of mycotoxins which may cause various ailments in humans and animals. Understanding the variability of the fungal population associated with head scab could improve disease control strategies. RAPD was used to study genetic variation in 15 isolates of *Fusarium graminearum*, collected from naturally infected wheat from Punjab, Tamil Nadu and high ranges of Himachal Pradesh during 2000–02. A screening of sixty-one 10-mer oligonucleotide primers (OPAA 1-20, OPAC 1-20, OPAD 1-20, OPV 14), revealed 19 RAPD primers which produced strong and reproducible DNA amplicons by PCR. The amplification products were in the range of 300 bp to 1.2 kb. Maximum number of bands (11) was obtained with primer OPAD 12 followed by ten bands with OPAA 12. Punjab isolates of *F. graminearum* from Gurdaspur (G 31) and Ludhiana (L23) were found genetically most similar (91.38%), whereas Wellington isolates of *F. graminearum* (W 5 and W 7) were found genetically most dissimilar (14.92%). Cluster analysis of band-sharing coefficients separated isolates of *F. graminearum* into four clusters. Lahaul valley isolates of *F. graminearum* (D 3, D 4 and D 5) grouped together (Group I), while *F. graminearum* isolates of Punjab

*For correspondence. (e-mail: mssaharan7@yahoo.co.in)

(G 31 and L 23) formed a separate group (Group II). *F. graminearum* isolates of Wellington, viz. W 1, W 2, W 5 and W 3, W 4, W 6, W 8 clustered in groups III and IV respectively.

Keywords: *Fusarium graminearum*, genetic variability, head scab, isolates.

FUSARIUM species are important pathogens of almost all cultivated crops. Mycotoxigenic *Fusarium* species cause significant quantitative and qualitative losses to global agriculture[1]. In addition, *Fusarium* species can cause serious animal and human diseases by producing harmful mycotoxins. Harvested grain is often contaminated with mycotoxins such as nivalenol (NIV), deoxynivalenol (DON) and zearalenone, which is unfit for consumption by poultry, humans and pigs[2–4]. Although several *Fusarium* spp. can cause head scab or head blight of wheat, *F. graminearum* Schwabe teleomporph *Gibberella zeae* (Schwein) Petch is primarily responsible for the recent epidemics in USA, China and other countries[5,6]. In the 1990s, scab caused an estimated US $3 billion loss to wheat and barley farmers in the US alone[7]. In India, *F. graminearum* causing ear blight and scab of wheat was reported from Arunachal Pradesh[8] and from Wellington, Tamil Nadu[9,10]. Losses of 15.1 to 29.0% in grain yield have been reported due to ear blight incidence on different wheat varieties from Arunachal Pradesh[11]. Maximum yield loss of 21.6% was recorded in wheat variety PBW 222 due to *Fusarium* head blight in Punjab[12].

Under the new WTO regime, wheat quality will be of paramount importance as this disease may hinder exports since *Fusarium* spp. are known to produce mycotoxins in grains. Wheat head scab is currently a disease of minor importance in India. However, due to global climate change there are chances of having more rainy days and high precipitation during the time of anthesis which will make wheat vulnerable to head scab. During 2005, due to continuous rain in March in Punjab, head scab appeared in a severe form on durum variety PDW 274 in Gurdaspur area. Panayotou *et al.*[13] have proposed that India will suffer severely from potential changes in temperature and precipitation. Due to lack of effective fungicides for controlling the disease, the development of resistant cultivars has become an important goal in breeding programmes of cereal crops[14]. For applying efficient strategies in the breeding process, knowledge about the genetic diversity and structure of naturally occurring pathogen populations is indispensable. *Fusarium* spp. identification by morphological characters like size, shape of conidia and pigmentation are highly dependent as these are influenced by cultural conditions. Considerable expertise is required to distinguish between closely related species and to recognize variations within the species[15]. Studies on molecular variation in *Fusarium* spp. are numerous[16,17]. Like in other pathogen systems, molecular techniques have become reliable and are highly suitable tools for identifying *Fusarium* species and for assessing genetic variation within collections and populations[18]. RAPD offers a promising, versatile and informative molecular tool to detect genetic variation within populations of plant pathogens[19]. Strains of *F. graminearum* have high degree of phenotypic variability. However, the genetic diversity of this fungus among Indian isolates has not been studied using DNA markers. The objective of the present study was to characterize *F. graminearum* isolates belonging to different locations of India at a molecular level.

Fusarium head blight (FHB) or head scab-infected samples were collected from naturally infected wheat earheads in Punjab, the high ranges of Himachal Pradesh and Wellington (Nilgiri Hills, Tamil Nadu) during 2000–02 (Table 1). Wheat seeds were surface-sterilized in 0.5% sodium hypochlorite for 1 min, rinsed in sterile distilled water, plated on potato dextrose agar[20] and stored at 4°C prior to use. Identification of *Fusarium* spp. was got confirmed by Indian Type Culture Collection (ITCC), Department of Plant Pathology, Indian Agricultural Research Institute, New Delhi.

Fungal cultures were grown in potato dextrose broth containing potato flakes (200 g), dextrose (20 g) in 1000 ml double distilled-water for 10 days at 25–28°C on a shaker. Lyophilized mycelia (250 mg) of 15 *F. graminearum* isolates which also showed pathogenic variability on a set of Indian wheat varieties were used for the study[21,22]. DNA was isolated from fresh mycelia using the CTAB method[23]. PCR amplifications were carried out in a total reaction volume of 25 µl comprised of 2 µl (20 ng) genomic DNA, 2.5 µl PCR buffer (10X) containing $MgCl_2$ (15 mM), 1 µl of 10 mM dNTP, 0.2 µl of Taq DNA polymerase (3 U/µl), and 2 µl (15 ng) each of oligonucleotide primer using a thermocycler (MJ Res.). PCR conditions consisted of DNA denaturation at 94°C for 2 min, 45 cycles of 1 min at 92°C, 1 min at 37°C and 2 min at 72°C. Amplification products were electrophoresed in 1.5% agarose gel in TAE buffer and visualized under UV light after staining with ethidium bromide. The assays were repeated at least thrice with each primer to confirm reproducibility of the amplification.

DNA bands that could be scored unequivocally for presence or absence were included in the analysis. Faint bands were not scored. The binary matrix was built pairwise and the presence or absence of the determined RAPD bands were scored as 1 and 0 respectively. The 100 bp DNA ladder was loaded in each lane on agarose gel to determine the band size. DICE coefficients were used to generate a dendrogram using the SAHN clustering program, selecting the unweighted pair-group method with arithmetic average (UPGMA) algorithm in NTSYS-pc[24]. The relationship among different isolates is given graphically in the form of a dendrogram.

We used RAPD to determine the genetic variability among 15 isolates of *F. graminearum* collected from dif-

Table 1. *Fusarium graminearum* isolates used in molecular analysis

Fusarium spp. (source of isolate)	Place of collection	Mountain/plain (altitude)	Collected from soil/plant	Year of collection
Fg (D3)	Lahaul valley (Himachal Pradesh)	High Himalayas (4000 m)	Wheat	2000
Fg (K4)	Lahaul valley (Himachal Pradesh)	High Himalayas (4000 m)	Wheat	2000
Fg (D4)	Lahaul valley (Himachal Pradesh)	High Himalayas (4000 m)	Wheat	2000
Fg (G31)	Gurdaspur (Punjab)	Foothills of Punjab (400 m)	Soil	2001
Fg (L23)	Ludhiana (Punjab)	Plains (300 m)	Soil	2001
Fg (G32)	Gurdaspur (Punjab)	Foothills of Punjab (400 m)	Wheat	2001
Fg (D5)	Lahaul valley (Himachal Pradesh)	High Himalayas (4000 m)	Wheat	2002
Fg (W1)	Wellington (Tamil Nadu)	Nilgiri Hills, Tamil Nadu (1850 m)	Wheat	2001
Fg (W2)	Wellington (Tamil Nadu)	Nilgiri Hills, Tamil Nadu (1850 m)	Wheat	2001
Fg (W3)	Wellington (Tamil Nadu)	Nilgiri Hills, Tamil Nadu (1850 m)	Wheat	2002
Fg (W4)	Wellington (Tamil Nadu)	Nilgiri Hills, Tamil Nadu (1850 m)	Wheat	2002
Fg (W5)	Wellington (Tamil Nadu)	Nilgiri Hills, Tamil Nadu (1850 m)	Wheat	2002
Fg (W6)	Wellington (Tamil Nadu)	Nilgiri Hills, Tamil Nadu (1850 m)	Wheat	2001
Fg (W7)	Wellington (Tamil Nadu)	Nilgiri Hills, Tamil Nadu (1850 m)	Wheat	2001
Fg (W8)	Wellington (Tamil Nadu)	Nilgiri Hills, Tamil Nadu (1850 m)	Wheat	2001

Fg, *Fusarium graminearum*; D, Dalang Maidan; K, Keylong; L, Ludhiana; G, Gurdaspur.

Table 2. RAPD primers used in the study of intraspecific variability of *F. graminearum* isolates

Primer	Sequence (5′ to 3′)	No. of amplicons	Size range of amplicon (bp)
OPAD-4	GTAGGCCTCA	7	600–2072
OPAD-5	ACCGCATGGG	6	800–1500
OPAD-10	AAGAGGCCAG	6	500–1700
OPAD-12	AAGAGGGCGT	11	500–2072, one band > 2072
OPAD-14	GAACGAGGGT	9	400–2072, one band > 2072
OPAD-15	TTTGCCCCGT	7	750–2072, one band > 2072
OPAA-7	CTACGCTCAC	7	650–2072, one band > 2072
OPAA-8	TCCGCAGTAG	6	800–2100
OPAA-9	AGATGGGCAG	5	300–1200
OPAA-12	GGACCTCTTG	10	400–2072, one band > 2072
OPAA-13	GAGCGTCGCT	9	450–2072, two bands > 2072
OPAA-16	GGAACCCACA	9	900–2072, four bands > 2072
OPAA-17	GAGCCCGACT	8	700–2072, one band > 2072
OPAC-3	CACTGGCCCA	5	750–2072, one band > 2072
OPAC-10	AGCAGCGAGG	6	900–2072, 2 bands > 2072
OPAC-11	CCTGGGTCAG	6	500–1700
OPAC-12	GGCGAGTGTG	5	1100–2072, one band > 2072
OPAC-17	CCTGGAGCTT	7	800–2072, one band > 2072
OPAC-19	AGTCCGCCTG	8	55–2072, one band > 2072

ferent regions of India. From the initial screening of sixty-one 10-mer oligonucleotide primers (OPAA 1–20, OPAC 1–20, OPAD 1–20, OPV 14), 19 informative primers were selected for analysis of all the isolates (Table 2). Primers which resulted in minimum five distinct bands were used for amplifying all the isolates. The level of polymorphism was different with different primers among different isolates (Figure 1). No primer alone could differentiate all the *F. graminearum* isolates.

The number of polymorphic bands generated by all RAPD primers and their sizes (bp) is given in Table 2. We detected most of the polymorphic fragments ranging from 2072 to 300 bp. Maximum number of bands (11) was scored with primer OPAD 12 followed by ten bands with OPAA 12 (Table 2). A minimum of five fragments were amplified with primer OPAA 9 (Table 2). The amplification products were in the range of 300 bp to 1.2 kb with this primer. An example of DNA fingerprints obtained with some RAPD primers is shown in Figure 1*a–f*. Multivariate analysis was conducted to generate a similarity matrix using DICE coefficient to estimate genetic diversity and relatedness among 15 *F. graminearum* isolates. Isolates from Punjab, viz. G 31 (Gurdaspur) and L 23 (Ludhiana) were genetically similar (91.38% similarity), while Wellington isolates (W 5 and W 7) were genetically dissimilar (14.92%). Coefficient of genetic similarity was calculated by the UPGMA method. These similarity coefficients were used to construct a dendrogram using

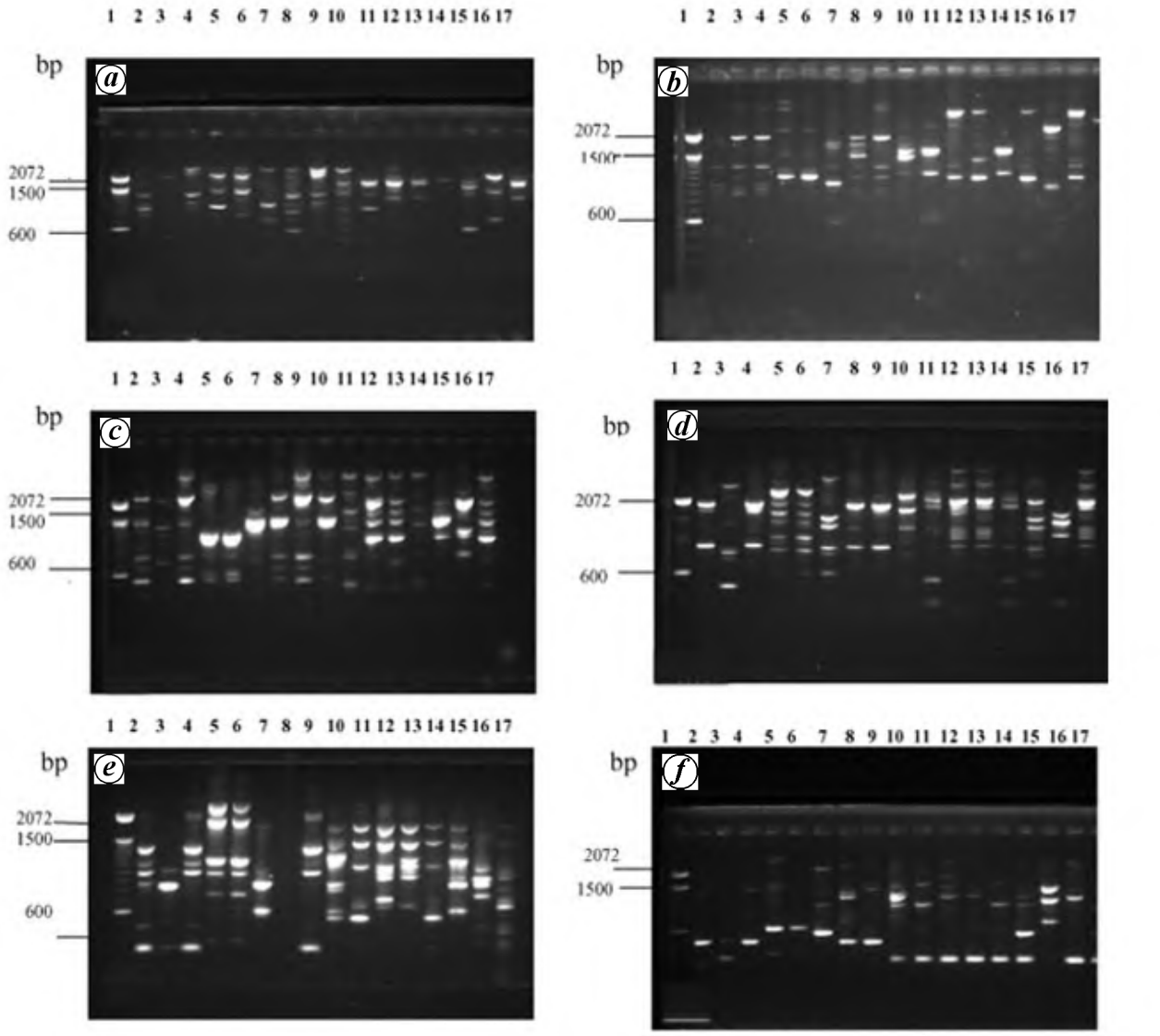

Figure 1. DNA fingerprint profiles of 15 isolates of *Fusarium graminearum* obtained with primers OPAC3 (*a*), OPAC10 (*b*), OPAA16 (*c*), OPAC19 (*d*), OPAD12 (*e*) and OPAD14 (*f*). Lane 1, 100 bp ladder; lanes 2–17, *F. graminearum* (Fg) isolates D3, K4, D4, G31, L23, G32, D5, W1, W2, W3, W4, W5, W6, W7, W8, respectively, except *F. semitectum* isolate in lane 7.

UPGMA analysis to determine the grouping of isolates (Figure 2). Coefficient of similarity among *F. graminearum* isolates ranged from 14.92 to 91.38%. The dendrogram obtained from cluster analysis of the DNA fingerprints revealed a great deal of heterogeneity among the isolates as forming four clusters. Out of four Lahaul valley isolates, three (D 3, D 4 and D 5) grouped together (Group I), while one (K 4) did not group in any cluster. *F. graminearum* isolates from Punjab (G 31 and L 23) formed a separate group (Group II). One isolate (W 7) collected from Wellington clustered along with *F. graminearum* isolates of Punjab (G 31 and L 23) in Group II. *F. graminearum* isolates from Wellington (W 1, W 2 and W 5) formed a group (Group III), while other isolates obtained from Wellington (W 3, W 4, W 6 and W 8) clustered in a separate group (Group IV).

This study has shown that there is considerable genotypic variability among *F. graminearum* isolates obtained from infected wheat earheads from different geographic regions of India. This variation may be because isolates belong to different geographic regions of India where the climate and farm practices and/or crop rotations vary greatly. There are differing reports in the literature on the genotypic diversity among the isolates of *F. graminearum*. Using PCR-based DNA fingerprinting, low genetic diversity among the isolates of *F. graminearum* was noted in Canada[25]. Genetic characterization of European isolates of *Fusarium* species associated with FHB revealed large variation among isolates of the *Fusarium* species[26,27]. Genetic diversity among isolates of *F. graminearum* in a recent study of 72 isolates from Queensland, Ontario and Prince Edward Island revealed that each isolate possessed a unique

DNA profile[28]. In *F. graminearum*, genetic variation can be introduced and maintained by mutation and somatic recombination of heterokaryons during asexual propogation via conidia. Furthermore, this species is homothallic and can undergo selfing and outcrossing with a high potential of recombination during meiosis leading to variation among ascospore propagules[29]. Using a large set of RAPD markers, high genetic diversity among *F. graminearum* isolates within fields and within heads of wheat has been observed in Germany[30].

RAPD has been successfully used to identify strains and races in phytopathogenic fungi[31,32]. It has also been used for studying inter- and intraspecific variability among populations from different as well as from the same geographic regions[33]. The RAPD pattern analysis showed variations at DNA level and is thus suitable for differentiation of *Fusarium* isolates below species level[19,25,34]. Traditional markers used to study the variability in plant pathogens are based on the differential hosts, cultural characteristics, morphological markers and biochemical tests. These markers distinguish pathogens on the basis of their physiological characters, i.e. pathogenicity and growth behaviour. However, these markers are influenced by host age, inoculum quality and environmental conditions. Moreover, these techniques are time-consuming, laborious and in some host–pathogen systems, including FHB of wheat, differential hosts are unavailable. In such cases, molecular markers are used for studying genetic variability in plant pathogens[35]. Using PCR, closely related strains of a pathogen can be distinguished without prior knowledge of the nature of polymorphic regions by the use of RAPD. PCR-based DNA fingerprinting, particularly with short oligonucleotide primers, has been used by various researchers for the analysis of genetic variation in plant pathogens[19].

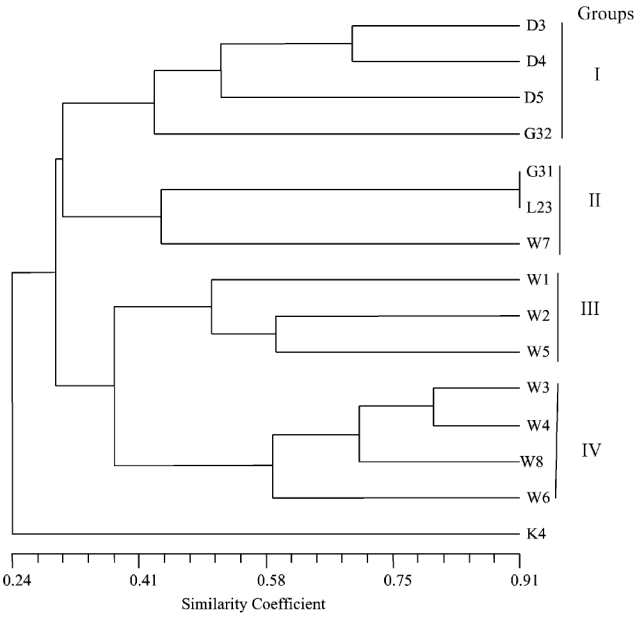

Figure 2. Dendrogram obtained from the combined dataset of 15 *F. graminearum* isolates with UPGMA based on DICE coefficient. Branches are labelled by isolate numbers. D, G, L, W and K represents *F. graminearum* isolates of Dalang Maidan, Gurdaspur, Ludhiana, Wellington and Keylong, respectively.

In the present study, Dalang Maidan isolates of *F. graminearum* (D 3 and D 4) which were found to be more aggressive in causing head scab on Indian wheat varieties clustered into one group, while Ludhiana and Gurdaspur isolates (L 23 and G 31), which were less aggressive[21], formed a separate group. Wellington isolates of *F. graminearum* also formed a separate group, though these isolates also caused severe effects on a set of wheat varieties compared to Punjab isolates of *F. graminearum*[21]. This large genetic variation detected at the DNA level indicates the ability of a pathogen to adapt to different life-cycle conditions. Since host resistance is quantitatively inherited in FHB, resistance genes of different origin should be pyramidized for effective management of disease.

1. Desjardins, A. E., Manadhar, H. K., Plattner, R. D., Maragos, C. M., Shrestha, K. and McCormick, S. P., Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. *J. Agric. Food Chem.*, 2000, **48**, 1377–1383.
2. Logrieco, A., Bottalico, A. and Ricci, V., Occurrence of *Fusarium* species and their mycotoxins in cereal grains from some Mediterranean countries. *Phytopathol. Mediterr.*, 1990, **29**, 81–89.
3. Marasas, W. F. O., Nelson, P. E. and Toussoun, T. A., *Toxigenic Fusarium Species: Identity and Mycotoxicology*, Pennsylvania State University Press, University Park, 1984.
4. Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U. S, Sugiura, Y. and Ueno, Y., Worldwide contamination of cereals by *Fusarium* mycotoxins nivalenol, deoxynivalenol and zearalenone. *J. Agric. Food Chem.*, 1988, **36**, 979–983.
5. Bai, G. H. and Shaner, G., Scab of wheat prospects for control. *Plant Dis.*, 1994, **78**, 760–765.
6. McMullen, M., Jones, R. and Gallenberg, D., Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Dis.*, 1997, **81**, 1340–1348.
7. Windels, C. E., Economic and social impacts of *Fusarium* head blight: Changing farms and rural communities in Northern Great Plains. *Phytopathology*, 2000, **90**, 17–21.
8. Roy, A. K., Ear blight and scab of wheat in Arunachal Pradesh (*Fusarium avenaceum*). *Curr. Sci.*, 1974, **43**, 162.
9. Brahma, R. N. and Singh, S. D., Occurrence of scab of wheat in the Nilgiri hills. *Curr. Sci.*, 1985, **54**, 1184–1185.
10. Saharan, M. S., Kumar, J., Sharma, A. K. and Nagarajan, S., *Fusarium* head blight (FHB) or head scab of wheat – a review. *Proc. Indian Natl. Sci. Acad. Sect. B*, 2004, **70**, 255–268.
11. Chaudhary, R. G., Edison, S. and Vishwadhar, Epidemiology and basic factors of severity, yield loss and grain quality deterioration due to ear blight of wheat in Arunachal Pradesh. *Indian J. Phytopathol.*, 1991, **43**, 571–574.
12. Kaur, S., Chahal, S. S. and Singh, N., Assessment of losses caused by head blight in wheat. *J. Mycol. Plant Pathol.*, 2000, **30**, 204–206.
13. Panayotou, T., Sachs, J. and Peterson, A., *Developing Countries and the Control of Climate Change: Empirical Evidence*, Centre for International Development, Harvard University, MA, USA, 1999.
14. Mesterhazy, A., Types and components of resistance to *Fusarium* head blight of wheat. *Plant Breed.*, 1995, **114**, 377–386.

15. Nelson, P. E., Toussoun, T. A. and Marasas, W. F. O., *Fusarium Species: An Illustrated Manual for Identification*, The Pennsylvania State University Press, University Park, 1983.
16. O'Donnell, K., Kistler, H. C., Tacke, B. K. and Casper, H. H., Gene genealogies reveal global phylogeographical structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 7905–7910.
17. O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C. and Aoki, T., Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genet. Biol.*, 2004, in press.
18. Burgess, L. W., Summerell, B. A., Backhouse, D., Benyon, F. and Levic, J., Biodiversity and population studies in *Fusarium*. *Sydowia*, 1996, **48**, 1–11.
19. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V., DNA polymorphisms amplified by arbitrary primers are useful genetic markers. *Nucleic Acids Res.*, 1990, **18**, 6531–6535.
20. Nirenberg, H. I., A simplified method for identifying *Fusarium* spp. occurring on wheat. *Can. J. Bot.*, 1981, **59**, 1599–1609.
21. Saharan, M. S., Kumar, J., Sharma, A. K., Tiwari, R. and Nagarajan, S., Pathogenic variation among *Fusarium* spp. associated with head scab of wheat in India. *Indian J. Agric. Sci.*, 2003, **73**, 322–326.
22. Sharma, A. K., Singh, D. P., Kumar, J., Singh, A. K., Saharan, M. S., Babu, K. S. and Shoran, J., Progress report of the coordinated experiments of crop protection (pathology and nematology). All India Coordinated Wheat and Barley Improvement Project, Directorate of Wheat Research, Karnal, 2004, p. 184.
23. Murray, M. G. and Thompson, W. F., Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.*, 1980, **8**, 4321–4325.
24. Rohlf, F. J., NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System, Version 2.01, Setauket, New York, 1998.
25. Ouellet, T. and Seifert, K. A., Genetic characterisation of *Fusarium graminearum* strains using RAPD and PCR amplification. *Phytopathology*, 1993, **83**, 1003–1007.
26. Nicholson, P., Jenkinson, P., Rezanoor, H. N. and Parry, D. W., Restriction fragment length polymorphism analysis of variation in *Fusarium* spp. causing ear blight of cereals. *Plant Pathol.*, 1993, **42**, 905–914.
27. Schilling, A. G., Moller, E. M. and Geiger, H. H., Polymerase chain reaction based assays for species specific detection of *F. culmorum*, *F. graminearum* and *F. avenaceum*. *Phytopathology*, 1996, **86**, 515–522.
28. Dusabenyagasani, M., Dostaler, D. and Hamelin, R. C., Genetic diversity among *Fusarium graminearum* strains from Ontario and Quebec. *Can. J. Plant Pathol.*, 1999, **21**, 308–314.
29. Burgess, L. W., Summerell, B. A., Bullock, S., Gott, K. P. and Backhouse, D., *Laboratory Manual for Fusarium Research*, University of Sydney, Australia, 1994, 3rd edn.
30. Schilling, A. G., Miedaner, T. and Geiger, H. H., Molecular variation and genetic structure in field populations of *Fusarium* species causing head blight in wheat. *Cereal Res. Commun.*, 1997, **25**, 549–554.
31. Chiocchetti, A., Ghignone, S., Minuto, A., Gullino, M. L., Garibaldi, A. and Migheli, Q., Identification of *Fusarium oxysporum* f. sp. *basilici* isolated from soil, basil seed and plants by RAPD analysis. *Plant Dis.*, 1999, **83**, 576–581.
32. Malvick, D. K. and Grau, C. R., Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the Midwestern United States. *Plant Dis.*, 2001, **85**, 740–744.
33. Walker, S. L., Leath, S., Hagler, W. M. and Morphy, J. P., Variation among isolates of *Fusarium graminearum* associated with *Fusarium* head blight in North Carolina. *Plant Dis.*, 2001, **85**, 404–410.
34. Grazal-Martin, M. J., Simon, C. J. and Muehlbauer, F. J., Use of random amplified polymorphic DNA (RAPD) to characterize race 2 of *Fusarium oxysporum* f. sp. *pisi*. *Mol. Plant Pathol.*, 1993, **83**, 612–614.
35. Sharma, T. R., Prachi, and Singh, B. M., Applications of polymerase chain reaction in phytopathogenic microbes. *Indian J. Microbiol.*, 1999, **39**, 79–91.

ACKNOWLEDGEMENTS. M.S.S. thanks Dr Gabriele Schachermayr, Programme Manager, Indo-Swiss Collaboration in Biotechnology; Prof. Genevie Defago, Federal Institute of Technology (ETH), Zurich, Switzerland, Dr S. R. Rao, Director, DBT, New Delhi; Project Director, DWR, Karnal and Dr A. K. Sharma, DWR, Karnal for support, guidance and encouragement during the course of investigation.

Received 6 March 2006; revised accepted 10 August 2006

Anti-inflammatory and antitumour activities of cultured mycelium of morel mushroom, *Morchella esculenta*

B. Nitha, C. R. Meera and K. K. Janardhanan*

Department of Microbiology, Amala Cancer Research Centre, Thrissur 680 555, India

Mushrooms are nutritionally functional food and a source of physiologically beneficial and non-toxic medicines. They have been used in folk medicine throughout the world since ancient times. *Morchella esculenta* (L) Pers. is an edible and highly priced mushroom. Commercial cultivation of this mushroom has not been successful till now and hence its mycelium is extensively used as a flavouring agent. Anti-inflammatory and antitumour activities of ethanolic extract of cultured mycelium of *M. esculenta* were investigated. The extract showed significant dose-dependent inhibition of both acute and chronic inflammation. The activity was comparable to that of the standard reference drug, Diclofenac. Antitumour activity of the extract was determined using both DLA cell line-induced solid tumour and EAC cell line-induced ascites tumour models in mice. The extract exhibited significant antitumour activity against both ascites and solid tumours. The finding suggests the potential therapeutic use of aqueous-ethanolic extract of morel mushroom mycelium in chemotherapy.

Keywords: Anti-inflammatory activity, antitumour activity, cultured mycelium, medicinal mushrooms, *Morchella esculenta*.

INFLAMMATION, a fundamental protective response, can be harmful in conditions such as life-threatening hyper-

*For correspondence. (e-mail: kkjanardhanan@yahoo.com)