disc around the ovary base is conspicuous with eight prominent lobes. Development of flower and fruit is comparatively slow in this taxon. The fruit in *X. mekongensis* is subglobose up to 10 cm across, with 10–15 pyramidal seeds, while in *X. granatum* the fruit is large, globose up to 20–30 cm across. Fruits and seeds and buoyant in both the taxa.

Mangroves are a valuable component of estuarine biodiversity. This natural ecosystem is exploited by both internal and external agents. The internal forces like the utilization of the mangroves by fisherman for timber, fuel, fodder and medicine is in practice since a long time even from prior to systematic identification of these taxa. External forces like large scale prawn culture practices are devastating these forests and the mangrove stretch is getting depleted day by day. Overexploitation is resulting in the disturbed distribution of some taxa, which are currently under pressure and these may end up in erosion unless conserved. One such taxon is *Xylocarpus*, which is exploited for its valuable timber. These taxa which were found in the Orissa coast earlier[5], disappeared in some regions according to a recent report[11] due to excessive felling. Presently a tree of these taxa with developed trunk is rarely found owing to felling. Sufficient number of plants of *X. mekongensis* is now found in this area, but *X. granatum* is very rare. During the entire survey I found about 10 plants of this taxon. Thus, there is an urgent need to conserve these precious mangrove tree taxa.

1. Hooker, J. D., *Flora of British India*, L. Reeve & Co., London, 1882, vol. I, pp. 566–567.
2. Gamble, J. S., *Flora of the Presidency of Madras*, BSI, Calcutta, 1957, (repr. edn), vol. 1, p. 132.
3. Tomlinson, P. B., *The Botany of Mangroves*, Cambridge University Press, Cambridge, 1986, pp. 274–282.
4. Singh, V. P. and Ajay Garge, *Ecology of the Mangrove Swamps of the Andaman Islands*, International Book Distributors, Dehradun, 1993, pp. 74–77.
5. Banerjee, L. K. and Rao, T. A., *Mangroves of Orissa Coast* (*and their ecology*), Bishen Singh Mahendra Pal Singh, Dehradun, 1990, pp. 48–51.
6. Banerjee, L. K., Sastry, A. R. K. and Nayar, M. P., *Mangroves in India: Identification Manual*, BSI, Calcutta, 1989, pp. 58–61.
7. Deshmukh, S. V., A global network of mangrove genetic resource centers – project formulation-workshop, Madras, 1991, pp. 15–25.
8. Blasco, F., *The Mangroves of India*, Inst. Francais, Pondicherry, *Trav. Sect. Sci. Tech*., 1975, **14**, pp. 128, 139, 140.
9. Rao, R. S., Sudhakar, S. and Venkanna, P., *Flora of East Godavari District*, INTACH, AP State Chapter, Hyderabad, 1999, pp. 258–259.
10. Sidhu, S. S., *Indian For*., 1963, **89**, 337–351.
11. Choudhury, B. P., Biswal, A. K. and Subudhi, H. N., *Rheedea*, (IAAT), 1991, **1**, pp. 62–67.

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Genetic diversity of *Colletotrichum graminicola* isolates from India revealed by restriction analysis of PCR-amplified intergenic spacer region of nuclear rDNA

Sorghum anthracnose, caused by *Colletotrichum graminicola* (Ces.) Wilson, is a destructive disease responsible for as high as 50% loss in grain yield[1]. Management of this disease through host plant resistance has often been unsuccessful due to the hyper-variable nature of this fungus[2]. A rapid and reproducible tool for characterizing the pathogen genotypes would help researchers follow the shift in genetic make-up of the pathogen population, thus providing a dynamic picture of the interactions between the host and pathogen genotypes. This would, in turn, help devising strategies for management of this disease. Genetic variability in this fungus was earlier studied by using molecular tools like RFLP and RAPD[3–7]. RFLP is a reliable tool, but is cumbersome, time-consuming and requires large amount of DNA. RAPD, on the other hand, is simple and rapid, but often not reproducible and error-prone[8]. Restriction analysis of the intergenic spacer region of the rDNA repeats has been useful for variability studies in some fungi like *Fusarium oxysporum*[9–11] and *Pyrenophora graminea*[12]. Once optimized (primer sequences and enzyme combinations), this technique combines the advantage of both PCR (simplicity and speed) and RFLP (reproducibility). The present communication reports on the successful use of the primer pair originally designed for *F. oxysporum*[9] and identification of a single restriction enzyme, *Kpn*I, which can be used for fingerprinting of *C. graminicola* populations.

The *C. graminicola* isolates were collected from six provinces of India where sorghum is cultivated widely (Table 1). Monoconidial isolates were grown in potato dextrose medium and DNA isolated, as described earlier[7]. For amplification of the intergenic spacer (IGS) region, primer pair CLN12 (5′CTGAACGCCTCTAAGTCAG3′) and CNS1 (5′GAGACAAGCATATGACTACTG3′), designed by Appel and Gordon[9] for *F. oxysporum* was used. Amplification conditions and other techniques were the same as described earlier[11]. Based on variation in the size of the IGS region, the isolates

Table 1. Isolates of *Colletotrichum graminicola* used in the present study

Isolates no.	Place of isolation	Cultivar
10B[a], 10C[a]	Shadnagar, Andhra Pradesh	Local
42A[a], 42B[a]	Patancheru, Andhra Pradesh	Breeding line
52B1	Kottakota, Andhra Pradesh	Local
131A	Patancheru, Andhra Pradesh	H112
22	Vadalgi, Maharashtra	CSH 9
88	Boti Bori, Maharashtra	CSH 9
89[a], 89B1[a]	Limba, Maharashtra	CSH 9
93	Kolambi, Maharashtra	Local
29	Udaipur, Rajasthan	Kakri local
141	Kanola, Rajasthan	Local
160	Chittorgarh, Rajasthan	Local
195	Areth, Gujarat	Local
35	Mircot, Gujarat	Local
123	Dharwad, Karnataka	DMS 652
124	Gulur, Karnataka	Fodder sorghum
48	Kovilpatti, Tamil Nadu	Local
158	Thamaraikulum, Tamil Nadu	Co 26

[a] Isolated from same lesion.

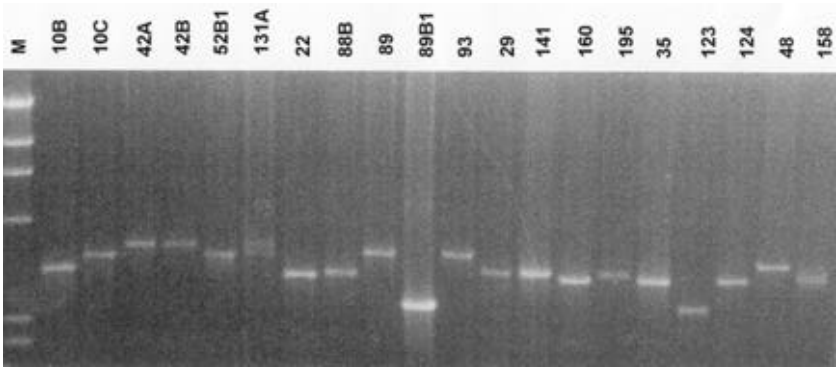

Figure 1. Size polymorphism in the PCR-amplified intergenic spacer (IGS) regions of *Colletotrichum graminicola* isolates. M denotes molecular weight marker (*l*-DNA digested with *Hin*dIII; Bangalore Genei, Bangalore).

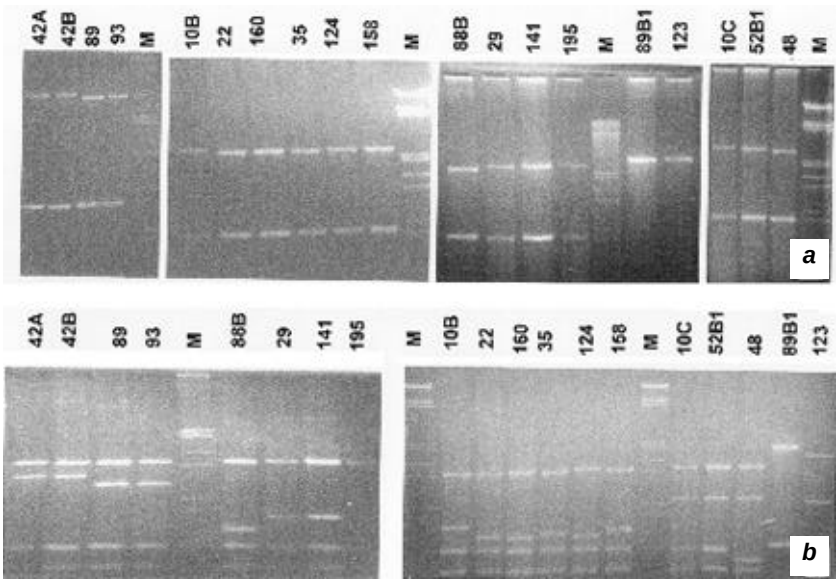

Figure 2. Restriction pattern (***a***) *Pst*I, and (***b***) *Kpn*I in the amplified IGS regions of different size-groups of *C. graminicola*. M denotes molecular weight marker (*l*-DNA digested with *Eco*RI and *Hin*dIII Bangalore Genei).

were first grouped into different size-groups and isolates from each size-group were analysed separately for restriction pattern. Initially, eight restriction endonucleases (*Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, *Kpn*I, *Sac*I, *Sal*I and *Xho*I), all hexa-base cutters, were used to study the number of sites in the *C. graminicola* isolates from a single group. Based on this result, *Kpn*I and *Pst*I were selected for studies with other groups. All the experiments were repeated at least twice with reproducible results.

PCR amplification of the IGS region with the primer pair CLN12 and CNS1 yielded single band (except for the isolate 131A, where a doublet could be seen) of 2.5–3.5 kb (Figure 1). Based on the size and number of amplicons, the 20 isolates could be grouped into six groups (group 1: 10C, 52B1, 48; group 2: 42A, 42B, 89, 93; group 3: 88, 29, 141, 195; group 4: 10B, 22, 160, 35, 124, 158; group 5: 89B1, 123; group 6: 131A). Analysis of the isolates from group 1 using eight restriction enzymes revealed no site for *Bam*HI, *Eco*RI, *Hin*dIII, *Sac*I, *Sal*I and *Xho*I in the IGS region. *Pst*I had a single site, while , *Kpn*I had three sites, thus yielding four fragments upon digestion of the amplified IGS region. Based on the restriction pattern with *Kpn*I or *Pst*I, isolates 10C and 52B1 could be differentiated from isolate 48 (Figure 2). Isolates from the other groups were then analysed using *Kpn*I or *Pst*I. Based on the polymorphism with respect to these sites in the IGS region, isolates 42A and 42B could be differentiated from isolates 89 and 93; isolate 88 from isolates 29, 141 and 195; isolates 89B1 from 123, and the isolates in group 4 could be subgrouped into three clusters: 10B, 22/160/124, and 35/158 (Figure 2). Based on these observations, it was felt that *Kpn*I digestion of the amplified IGS region is a good indicator of genetic polymorphism in *C. graminicola*. We, therefore, digested the IGS region for all the 20 isolates with *Kpn*I and the digestion pattern revealed that this could be used as a sound fingerprinting technique for this pathogen.

A further analysis of grouping *vis-à-vis* source of the isolates (Figure 3) revealed an interesting trend – there was no relation between the genotype and source (location/cultivar). For example, isolates from western India grouped with the ones from southern India, even in cases where the provinces do not share a com-

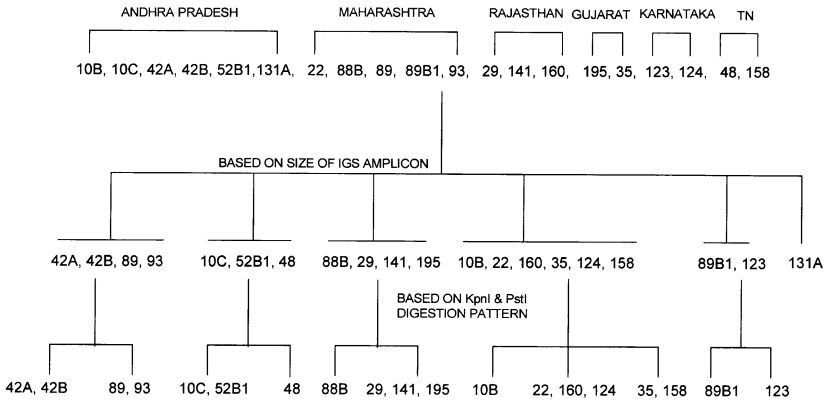

Figure 3. Classification of *C. graminicola* isolates based on origin, size and restriction polymorphism in the IGS region.

mon border (e.g. isolate 35 from Gujarat was similar to isolate 158 from Tamil Nadu). On the other hand, isolates from single lesion (10B to 10C and 89 to 89B1) were distinctly different from each other.

1. Ali, M. E. K. and Warren, H. L., in *Sorghum and Millet Diseases: A Second World Review* (eds de Milliano, W. A. J., Frederiksen, R. A. and Bengston, G. D.), International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 1992, pp. 203–208.
2. Rao, V. P., Thakur, R. P., and Mathur, K., *Indian Phytopathol*., 1998, **51**, 164–174.
3. Guthrie, P. A. I., Magill, C. W., Frederiksen, R. A. and Odvody, G. N., *Phytopathology*, 1992, **82**, 832–835.
4. Rosewich, U. L., Pettway, R. E., McDonald, B. A., Duncan, R. R. and Federikson, R. A., *Phytopathology*, 1998, **88**, 1087–1193.
5. Backman, P. A., Landschoot, P. J. and Huff, D. R., *Crop Sci*., 1999, **39**, 1129–1135.
6. Browning, M., Rowley, L. V., Zeng, P. Y., Chandlee, J. M. and Jackson, N., *Plant Dis*., 1999, **83**, 286–292.
7. Latha, J., Mathur, K., Mukherjee, P. K., Chakrabarti, A., Rao, V. P. and Thakur, R. P., *Indian Phytopathol*., 2002, **55**, 19–25.
8. Dyer, A. T. and Leonard, K. J., *Phytopathology*, 2000, **90**, 565–567.
9. Appel, D. J. and Gordon, T. R., *Phytopathology*, 1995, **84**, 786–791.
10. Alves-Santos, F. M., Benito, E. P., Eslava, A. P. and Diaz-Minguez, J. M., *Appl. Environ. Microbiol*., 1999, **65**, 3335–3340.
11. Chakrabarti, A., Mukherjee, P. K., Sherkhane, P. D., Bhagwat, A. S. and Murthy, N. B. K., *Curr. Sci*., 2001, **80**, 571–575.
12. Pecchia, S., Mercatelli, E. and Vannacci, G., *FEMS Microbiol. Lett*., 1998, **166**, 21–27.

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