protein bodies enlarge and turn into vacuoles as their contents are progressively digested (Figure 3 *g*). The vacuoles then fuse together and form one large vacuole per cell. This process can be observed not only in the scutellar parenchyma cells but also in all embryonal organs. The scutellar epithelial cells contain small protein bodies. The epithelium secretes enzymes into the starchy endosperm to digest the macromolecules, and reabsorbs low molecular weight substances to be transported to the embryo (Figure 3 *e*). Ungerminated embryo does not contain any starch (Figure 2 *b*). During germination, within 12 h of imbibition of water, starch deposition begins in the embryo and continues for several days.

The endosperm is primarily a storehouse of starch (Figures 1 *c*, 2 *a*, *b*, *e*, 3 *e*). However, each dead endosperm cell retains the remnants of a triploid nucleus. The DNA-specific fluorochrome DAPI reveals the nuclear material in the endosperm cells (Figure 3 *h*, *i*). Obviously the starchy endosperm can also make a significant contribution of nitrogen bases to the germinating embryo and human nutrition. The starchy endosperm also contains proteins (Figure 2 *g*, *h*). Most of the proteins occur in the subaleurone layers (Figure 2 *g*, *h*). Fluorochromes specific for proteins (8-anilino-1-naphthalenesulfonic acid and dansylchloride) as well as non-specific fluorochromes such as barbituric acid, aniline blue, acridine orange and calcofluor white M2R can be used to detect proteins in the endosperm. Proteins are stored in discrete structures known as protein bodies (PB). The spherical PB I stores prolamins and the slightly larger, irregularly shaped PB II stores glutelins and globulins[12].

A survey of the wild species of rice and grains stored for more than 12 years reveals the same pattern of distribution of all storage substances, including the nuclear remains and minerals detected in IR50.

This histochemical survey of rice caryopsis provides a broad framework of reference for the understanding of time and place of deposition of storage material within the caryopsis, as well as its removal during seed germination. The emerging picture of the rice grain will help biotechnologists to sharpen their focus on spatial and temporal events for genetic manipulation to improve grain quality. Genetic manipulation altering the quality of rice lipids should focus on the aleurone as well as the embryo since these are the major tissues that store lipids. The subaleurone layers should be the major target for controlling the expression of protein genes for quality and quantity enhancement in transgenic plants. The deeper layers of the endosperm could be manipulated to promote deposition of more proteins so that the total protein content of the grain can be increased. Enhancement of carotene content may be attempted by the manipulation of cross cells and the embryonal tissue which possess plastids/proplastids. Structural and histochemical investigations will continue to complement the efforts of researchers interested in all aspects of improvement of rice.

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A simple and rapid molecular method for distinguishing between races of *Fusarium oxysporum* f.sp. *ciceris* from India

Apratim Chakrabarti, Prasun K. Mukherjee*, Pramod D. Sherkhane, Anjali S. Bhagwat and Narra B. K. Murthy

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India

***Eco*RI restriction pattern of the nuclear ribosomal DNA from four isolates of *Fusarium oxysporum* f.sp. *ciceris* (FOC) representing four races prevalent in India indicated that the races could be grouped into three distinct groups; races 1 and 4 representing one group and race 2 and race 3, the other two. The restriction pattern indicated presence of three *Eco*RI sites on the nuclear rDNA of this species, one each on the 5.8S and the 25S regions, conserved to all, and the other one, i.e. the variable site, on the intergenic spacer (IGS) region of the nuclear rDNA. The same was confirmed by PCR-amplification of the IGS region followed by digestion with *Eco*RI and a set of other enzymes. It is suggested that amplification of the IGS region and digestion with restriction enzymes could be used to study polymorphism in FOC, and to rapidly identify the races existing in India. We also propose that out of the four types of races described from India, races 1 and 4 are the same.**

FUSARIUM oxysporum Schl. Fr. f.sp. *ciceris* (Padwick) Mauto & Sato, which incites wilt, is one of the major

*For correspondence. (e-mail: pppr@magnum.barc.ernet.in)

pathogens of chickpea (*Cicer arietinum* L.) worldwide, especially in the Indian subcontinent and the Mediterranean basin[1,2]. The best available method for control of fusarial wilt is the cultivation of resistant chickpea varieties[3]. However, the existence of several physiological races of the pathogen makes it complicated to contain this disease through resistance breeding. Seven races of this pathogen have so far been reported; races 1–4 from India; 0, 1, 5, 6 from California and Spain; 1 and 6 from Morocco and 0 from Tunisia[4–6]. Race 1 has further been subdivided into three sub-groups, viz. 1A, 1B and 1C[6]. Of these races, race 1A, 2 through 6 cause typical fusarial wilt and necrosis, whereas races 0, 1B and 1C induce yellowing syndrome in the absence of wilting[6]. The races are conventionally identified by inoculation of a set of 10 differential varieties[3]. This method of race identification is expensive, time consuming (at least 40 days) and may be influenced by variability inherent in the experimental system[7–9]. In this regard, a rapid molecular method, therefore, would be advantageous over conventional techniques of race identification.

Using RAPD/AFLP analysis, Kelley *et al.*[10,11] could differentiate between the two groups/pathotypes inducing either wilt or yellowing symptoms. No polymorphism in the mitochondrial DNA was detected among the seven races studied by Perez-Artes *et al.*[12]. RFLP analysis of the nuclear ribosomal DNA (rDNA) was useful in demonstrating species-specific differences in toxin-producing *Fusarium* spp.[13]. Appel and Gordon[14] detected inter-strain variability in *F. oxysporum* f.sp. *melonis* by RFLP analysis of the amplified intergenic spacer (IGS) region, and subsequently by partial sequencing of the IGS region[15]. In PCR-amplified IGS region, RFLP variation was also detected in other fungi like *Histoplasma capsulatum*[16] and *Puccinia graminis*[17]. The present paper reports on the genetic variability in the wilt-causing pathotype of *F. oxysporum* f.sp. *ciceris* (FOC) using molecular methods based on the variability in rDNA region. The usefulness of this method in studying the polymorphism in the wilt-inciting isolates of FOC and the rapid race identification has been discussed.

Four isolates of FOC representing four races from India were collected from the International Crops Research Institute for the Semi-Arid Tropics, Patancheru. Race 1 was originally isolated from Hyderabad, 2 from Kanpur, 3 from Gurdaspur and 4 from Jabalpur. The cultures were multiplied and maintained on potato dextrose medium.

High molecular weight genomic DNA from *Fusarium* mycelium grown in potato dextrose broth for 3 days, was isolated using a method described by Kim *et al.*[18]. Briefly, the mycelium was harvested on Whatman no. 1 filter papers, washed thoroughly and ground in liquid nitrogen. Five ml of extraction buffer (100 mM NaCl, 10 mM Tris, 1 mM Na_2EDTA, pH 8) containing 1.0% SDS and 0.5% β-mercaptoethanol was added to 2 g of ground mycelia taken in 50 ml Sorvall centrifuge tubes and mixed thoroughly. The mixture was incubated at room temperature for 30 min and extracted twice with equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1) and twice with chloroform/isoamyl alcohol. DNA was precipitated with two volumes of ice-cold ethanol, spooled, washed with 70% ethanol and dissolved in TE buffer (10 mM Tris, 1 mM Na_2EDTA, pH 8). DNA was digested to completion with the restriction enzyme *Eco*RI (Bangalore Genei, India) as per the manufacturer's specifications. The restriction fragments were size-separated by electrophoresis in 0.8% agarose gel and transferred onto positively charged nylon membrane (Boehringer Mannheim) by alkaline transfer method[19]. The *Neurospora crassa* rDNA repeat unit was purified from plasmid pMF2 (kindly supplied by P. J. Russell) as a 6.3 kb *Pst*I fragment[20] and labelled with [α-^{32}P]dATP and [α-^{32}P]dCTP using a random priming kit supplied by Board of Radiation and Isotope Technology, Mumbai, according to the supplier's specifications. Hybridization was performed with the labelled probe at 65°C overnight, and stringency washes given as per standard protocols[19].

Amplification of the ITS1–5.8S–ITS2 region of the nuclear rDNA was performed using the primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) according to White *et al.*[21]. The

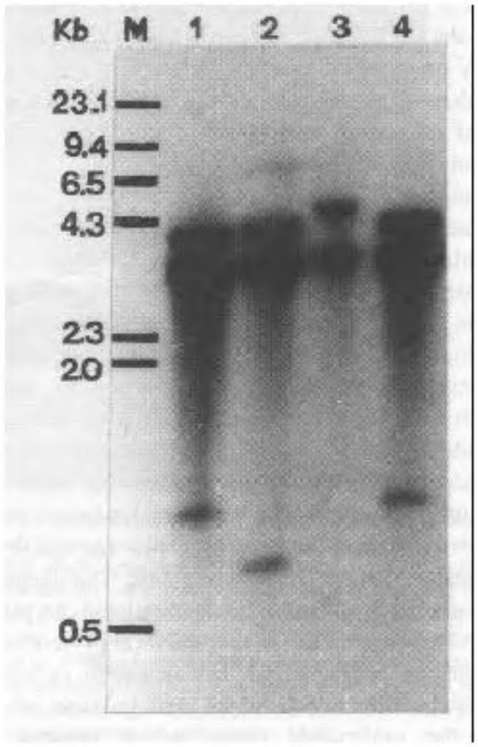

Figure 1. Restriction (*Eco*RI) pattern in the nuclear ribosomal DNA of four races of FOC with rDNA from *N. crassa* as probe. Lane 1, Race 1; Lane 2, Race 2; Lane 3, Race 3; Lane 4, Race 4; and M, Mol. wt. Marker (λ-*Hin*dIII digest).

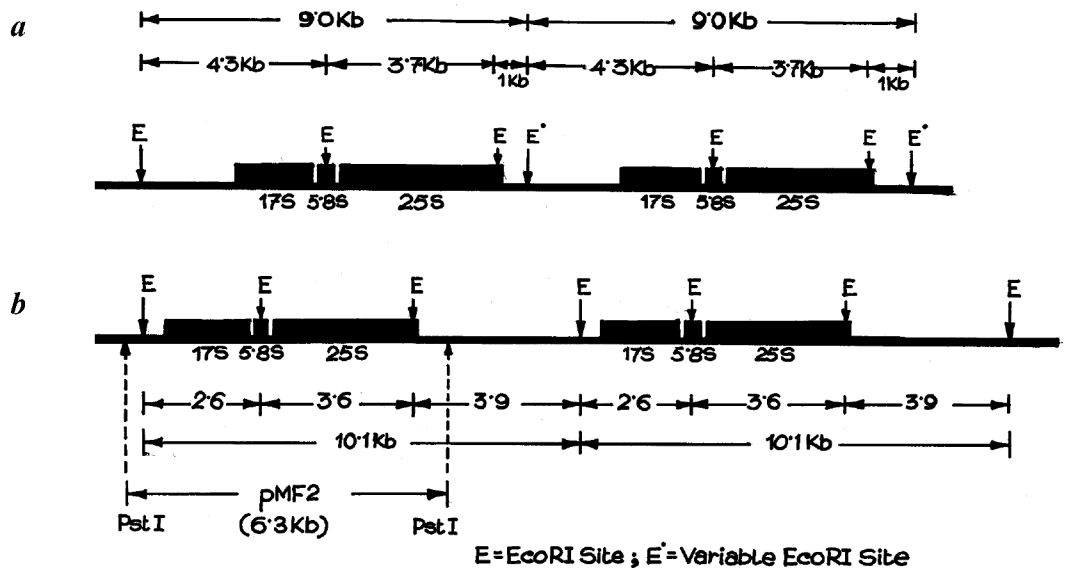

Figure 2. *Eco*RI restriction map of nuclear rDNA of (*a*) FOC compared to (*b*) *N. crassa* (after Russel *et al.*[20]).

amplified product was digested with *Eco*RI and size-separated on 1.5% agarose gel. Amplification of the IGS region was performed using the primer pair CLN12 (CTGAACGCCTCTAAGTCAG) and CNS1 (GAGACAAGCATATGACTACTG) designed by Appel and Gordon[15] for *F. oxysporum* with priming sites at the 3′ end of the *28S* gene and 5′ end of the *18S* gene, respectively. The amplification mix contained 0.5 µM primer, 10 ng DNA, 0.1 mM of each of the dNTPs in 1X reaction buffer containing 1.5 mM $MgCl_2$. After initial melting at 95°C for 5 min, temperature was held at 68°C and 1U of *Taq* polymerase (Bangalore Genei, India) was added per 25 µl final volume of reaction mix. This was followed by 30 cycles of amplification with the following parameters: annealing at 55°C for 1 min, extension at 72°C for 2 min, melting at 94°C and a final extension of 10 min at 72°C. The amplified products were extracted twice with chloroform/isoamylalcohol (24:1), ethanol precipitated, washed with 70% ethanol and dissolved in TE. The products thus purified were digested separately with *Eco*RI, *Bam*HI, *Hin*dIII, *Pst*I, *Sac*I (New England Biolabs), *Taq*I (Boehringer Mannheim), *Alu*I and *Sau*3AI (Bangalore Genei, India) as per manufacturers' instructions, and size-separated on 1.5% agarose gel as described above.

Three distinct *Eco*RI restriction patterns were observed for the tandemly repeated rDNA of different races of FOC (Figure 1). Hybridization of *Eco*RI digested genomic DNA of FOC with the *N. crassa* rDNA yielded three fragments. The restriction pattern for races 1 and 4 was identical, while it was different for races 2 and 3. All the isolates tested had a common 3.7 kb fragment in the middle, but the variation was in the upper (4.3–4.7 kb) and the lower (0.6–1 kb) bands.

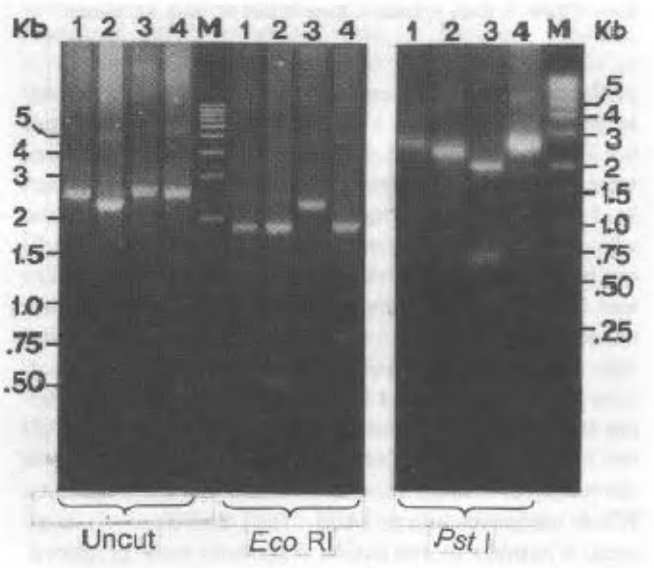

Figure 3. PCR amplified IGS region and its *Eco*RI and *Pst*I restriction patterns of four races of FOC. Lane 1, Race 1; Lane 2, Race 2; Lane 3, Race 3; Lane 4, Race 4; and M, Mol. wt. marker.

Digestion of the amplified ITS1–5.8S–ITS2 region with *Eco*RI revealed an *Eco*RI site in the 5.8S region (data not presented), which seems to be conserved in filamentous fungi, including *N. crassa*[20]. Combining these two data, and comparing with the *N. crassa* rDNA map, we could locate the variable *Eco*RI site on the IGS region (Figure 2).

Using the primer pair CLN12 and CNS1, we could amplify the IGS region from all the 4 races, the approximate size being 2.6 kb (2.4 for race 2). Digestion

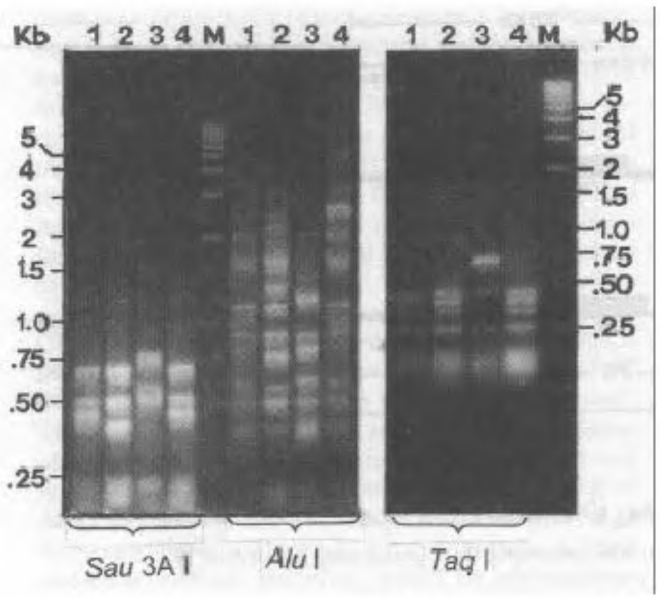

Figure 4. Restriction pattern of PCR amplified IGS region of four races of FOC with *Alu*I, *Sau*3AI and *Taq*I. Lane 1, Race 1; Lane 2, Race 2; Lane 3, Race 3; Lane 4, Race 4; and M, Mol. wt. marker.

of the amplified IGS region with *Eco*RI produced similar bands for both race 1 and race 4 (one 1.9 and another 0.7 kb). But individual and distinctive banding patterns were observed for both race 2 (1.9 and 0.5 kb) and race 3 (2.2 and 0.4 kb). This conclusively proved that the variability in the restriction patterns observed in Southern hybridization with rDNA repeat unit from *N. crassa* was indeed due to variability of the *Eco*RI site within the IGS region. When variability within the IGS region was further studied using *Hin*dIII, *Bam*HI and *Sac*I, none of them restricted the amplified fragment indicating the absence of these sites within IGS region in all the four races. However, *Pst*I was found to have one site only in case of race 3 (2.0 and 0.6 kb fragment). When tetrabase cutters (*Alu*I, *Taq*I and *Sau*3AI) were used, a number of restriction fragments were produced. But in each case races 1 and 4 had similar profiles, whereas those for races 2 and 3 were always different from each other and the rest (Figures 3 and 4).

FOC races were originally classified based on wilt reaction on 10 differential lines[4]. The disease reaction was scored based on per cent wilting (resistant 0–20%, moderately susceptible 21–50%, and susceptible ≥ 51% wilt). While races 1–3 could be clearly identified based on distinct reactions (either resistant or susceptible) on two differential lines, race 4 was designated based on reaction on only one differential line, i.e. CPS-1, and the reaction was 'moderately susceptible' (21–50% wilting) compared to 'resistant' (0–20% wilting) for race 1. This observation is indicative of relatedness of race 1 to race 4. The data obtained in the present experiment also indicate that races 1 and 4 are identical with respect to restriction pattern of the IGS region of the rDNA, even with tetrabase cutters. We therefore propose that out of the four races described based on differential reactions (which is subjected to environmental and experimental variations), races 1 and 4 are the same. Race 1 or 4 is, however, distinctly different from race 2 and race 3, which are different from each other. The present investigation indicates that the wilt-causing isolates of FOC are genetically distinct and polymorphism can be studied in the wilt causing pathotype using the variability in the IGS region. We also suggest that after further verification with more representative isolates, it could be possible to develop a simple, rapid and reliable method of race identification in FOC by amplifying the IGS region and restriction digestion of the same with appropriate restriction enzymes.

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Has the frequency of intense tropical cyclones increased in the north Indian Ocean?

O. P. Singh*, Tariq Masood Ali Khan and Md. Sazedur Rahman

SAARC Meteorological Research Centre, Abhawa Bhaban, Agargaon, Dhaka 1207, Bangladesh

An effort has been made to settle the question whether the intense cyclones have become more frequent over the north Indian Ocean, posing a more serious threat to the vulnerable coastal population of the region. The results of the study, which has considered the entire existing data of 122 years of tropical cyclone frequency over the north Indian Ocean from 1877 to 1998, have shown that there is indeed a trend in the enhanced cyclogenesis during November and May. These months account for the maximum number of severe cyclones over the north Indian Ocean. The increasing trend in the cyclone frequency during these months has been primarily due to the significant positive trends over the Bay of Bengal, where the majority of north Indian Ocean cyclones develop. Thus the coastal regions of Bangladesh, India and Myanmar have indeed become more prone to the incidence of severe cyclones during November and May.

There has been a two-fold increase in the tropical cyclone frequency over the Bay of Bengal during November in the past 122 years. There has been a 17% increase in the intensification rate of cyclonic disturbances to the cyclone stage, and a 25% increase to severe cyclone stage over the north Indian Ocean during November, which accounts for highest monthly average of severe cyclone frequency. All these linear trends are statistically significant at 99% level. The increasing trend in the cyclone frequency during May is also highly significant but the intensification rates to cyclone and severe cyclone stages have registered only slight increasing tendencies. The cyclonic frequencies during transitional monsoon months, June and September, have diminished considerably. The detailed results have been presented for November and May only.

TROPICAL cyclones are among the most destructive natural disasters of the world. The north Indian Ocean accounts for 7% of global tropical cyclones[1]. More cyclones form in the Bay of Bengal than the Arabian Sea; the ratio of their respective frequencies is about 4:1 (ref. 2). There are two cyclone seasons in the north Indian Ocean, viz. pre-monsoon (especially May) and post-monsoon (especially October and November). A few cyclones form in transitional monsoon months June and September also. On an average about 5–6 tropical cyclones (maximum sustained wind of 34 knots or more) form in the Bay of Bengal and the Arabian Sea every year, of which 2–3 reach severe stage (maximum sustained wind of 48 knots or more). The total number of tropical cyclones in the Bay of Bengal and the Arabian Sea during May, June, September, October and November is given in Table 1.

The socio-economic impact of tropical cyclones is considerable[3]. The coasts of India, Bangladesh and Myanmar suffer enormous loss of life and property every year due to cyclones in the Bay of Bengal. Due to the high population density in the coastal regions, Bangladesh is most vulnerable to the hazards of tropical cyclones[4]. Therefore, any change in the tropical cyclone frequency in the Bay of Bengal would have far reaching consequences in the countries surrounding the Bay of Bengal rim.

The assessment of climate change effects on tropical cyclones is necessary, both in terms of occurrences and tracks. Cyclone activity may be affected by the changes in sea surface temperature (SST). For instance, El-Nino/Southern Oscillation is known to influence cyclone frequency in different ocean basins[5–7]. Therefore, the impacts of long-term SST trends on the cyclone frequency in each ocean basin needs to be documented. Some investigators have studied the changes in the

Table 1. Frequency of tropical cyclones in the Bay of Bengal and the Arabian Sea during the period 1877–1998

	Month				
	May	June	September	October	November
Bay of Bengal					
Cyclonic storms	59	35	40	89	114
Severe cyclonic storms	42	5	16	38	63
Arabian Sea					
Cyclonic storms	24	25	4	24	20
Severe cyclonic storms	19	17	2	11	15

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