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Genomic Variability Among *Moesziomyces penicillariae* Populations in Senegal (West Africa)

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ABSTRACT

The genomic diversity of sixty three isolates of *Moesziomyces penicillariae*, the causal agent of smut of Pearl millet, was analysed using an ISSR (Inter Single Sequence Repeats) approach based on the universal minisatellite probe M13 [GAGGGTGGCGGTTCT (Karlsson, 1994)]. According to the electrophoretic patterns, the distance tree obtained indicates that the different genotypes from Senegal are divided into 4 clades. This genetic differentiation is correlated to the geographical origin of the isolates. Most of the isolates from the Northern areas formed a separate clade as well as those from the south. The isolate from Gambia is closer to those of the Southern of Senegal whereas those of Burkina formed an independent clade. The isolates from the Central and Eastern areas are distributed in two clades. However, in these regions, a higher genetic mix appeared: the distribution of the genotypes is not strictly correlated to their geographic origin. Our results point out that two major factors are involved in the geographic distribution of the populations of *M. penicillariae* in Senegal. Firstly, the genetic diversity is partly correlated to the climatic conditions in relationship to the Pearl millet varieties used in the different areas. Secondly, isolates sharing genetic markers were collected along the commercial ways, revealing that anthropochory has an important incidence in dispersal of the disease. These results highlight that the incidence of the smut disease of Pearl millet in Senegal must be controlled by using tolerant varieties adapted to the different climatic conditions and by the application of sanitary rules limiting the dispersal of infected seeds.

Key words: Pearl millet smut, *Moesziomyces penicillariae*, genomic diversity, Senegal

INTRODUCTION

Moesziomyces penicillariae (Brefeld) Vànky, the causal agent of Pearl millet smut, is a fungus belonging to Ustilaginaceae family (Basidiomycota). The fungus converts the ovaries of Pearl millet to a sori filled of teliospores. This disease has been reported in the tropical and subtropical areas of Africa, America and Asia (Wells, Burton & Ourecky 1963, Thakur & King, 1988). In spite of the use of tolerant varieties resulting from plant breeding (see www.icrisat.org, Sangwan & Thakur 1981, Thakur *et al.*1986), smut is still one of major diseases of Pearl millet in sub-sahalian areas. The severity of the symptoms is highly dependent on moisture at time of flowering (Bhatt, 1946, Thakur & King, 1988, personal observations) but also on the temperature (Thakur & King, 1988, personal observations). High variations in smut incidence were observed on same tolerant varieties of pearl millet in various sites of survey [Hisar, Jamnagar, Patancheru (India), Bambey (Senegal), Samaru (Nigeria), Kamboinse (Burkina Faso), Sadore (Niger)] (Williams, 1984). As underlined by Thakur *et al.* (1986), these variations of tolerance can be due to the genetic variability of the pathogen.

Wilson and Bondari (1990), in a first study on variability of *M. penicillariae* isolates, reported that pathogenicity of strains could vary according to their morphological types. Many authors described that smut fungi present a high morphologic polymorphism (Cherewick, 1958, Holton, Hoffmann &. Duran 1968) but no strict correlation with virulence was demonstrated. However, these results suggest that different sub-populations of *M. penicillariae* exist and could support the variation of tolerance observed on pearl millet varieties.

In this study, we performed a large collection of isolates in different regions of Senegal. Strains collected in Burkina and Gambia were used as outgroup references. We performed the genomic printing of these isolates in order to obtain an overview of the genetic variability of the strains from Senegal. The strategy used is based on an ISSR (Inter Single Sequence Repeat) approach using minisatellite sequences. Minisatellites sequences are strongly polymorphic sequences and their allelic variability can be exploited to simultaneously identify several highly variable loci of the genome. We used the minisatellite probe M13 (Brocas, *et al.* 1987, Vassart *et al.* 1987). This sequence allowed the detection of highly variable regions in animal genome as well as in plants and fungi (Ryskov, *et al.* 1988, Meyer & Mitchell 1995, Gadkar, Adholeya & Satyanarayana 1997, Olive & Bean 1999, De la Puente-Redondo *et al.* 2000, Zehdi *et al.* 2004). The objective of this study is not to characterise virulent

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strains, but to obtain a first analysis of the genetic diversity of *M. penicillariae* strains in order to elaborate stronger strategies to limit the incidence of smut of Pearl millet.

MATERIALS AND METHODS

Fungal material

Naturally infected ears of Pearl millet were harvested in different areas of Senegal. Intact sori were collected on *Pennisetum glaucum* (L.) R. Br. var. "souna3", "souna local" and "sanio", on wild millet *Pennisetum violaceum* and on "chibra", a hybrid of *P. glaucum* and *P. violaceum*. Samples were then kept into Petri dishes at room temperature. To avoid the interference of contaminant DNA from plants and microorganisms, ISSR experiments were carried on multisporidial cultures issuing from each sorus. Cultures were isolated by following some modifications of the methods described by Wells, Hanna & Burton (1987) and the data sheet of WADMSON service (2001). Sori with intact sheaths were surface disinfected by shaking during 10 minutes into a 2% aqueous solution of chloramine T (Sigma) with a drop of Tween 80, then rinsed three times into sterile distilled water. The sori were then crushed aseptically to release the teliospores into small sterile Petri dishes. Teliospores resulting from each sorus were placed on PDA medium (Potato Dextrose Agar; Difco) for germination at 28°C. After 8-10 days, the resulting yeast colonies were picked up in microtubes of 1.5 ml. They were washed 3 times with 1 ml of UHQ water, centrifuged and preserved at –20°C. Each culture constitutes an original multisporidial isolate (mix of sporidia resulting from several teliospores of one sorus). Sixty isolates were thus selected in four zones of Senegal: 16 in the Northern zone, 25 in the Central zone, 7 in the Eastern, 12 in the Southern zone; also one isolate from Gambia and two from Burkina Faso (Table 1).

Molecular analysis

The genomic DNA was extracted from each isolate using the CTAB procedure (Gardes & Bruns, 1993). PCR protocol includes the use of a proof reading TaqPolymerase (Clontech). The presence of contamination in each DNA solution has been checked by amplification with the universal primers pITS1 and pITS4 (White *et al*., 1990) allowing the amplification of the ribosomal sequences ITS1-5,8S-ITS2. ITS of *M. penicillariae* are 750bp length. PCR amplifications were performed as follow: 4 minutes at 94°C, 35 cycles (1 min at 54°C, 1 min at 72°C and 1 min at 94°C), 10 min at 72 °C. After the purity of the DNA extract was assessed. The minisatellite primer M13

Table (1): Collection of *M. penicillariae* isolates. Sites of harvest, corresponding name of the isolate and hosts.

Northern areas	isolate/host	Southern areas	isolates/host	Central areas	isolates/host	Eastern areas	isolates/host
Rao	**N1**/Souna	Mpack	**S1**/Sanio	Bambey sérère	**C1**/Souna 3	Tambacounda	**E1**/Souna
Mpal	**N2**/Souna	Kolda	**S2**/Sanio	Bambey sérère	**C2**/P.Chibra	Koussanar	**E2**/Souna
Mpal	**N3**/*P.violaceum v*	Sandignéri	**S3**/Sanio	Khombole	**C3**/Souna	Koumpentoum	**E3**/Souna
Mpal	**N4**/*P.violaceum f*	Sédhiou	**S4**/Souna	Thiès	**C4**/Souna	Koumpentoum	**E4**/Sanio
Mpal	**N5**/*P.violaceum f*	Sédhiou	**S5**/Sanio	Pouyenne	**C5**/Souna	Sinthiou	**E5**/Guériniari 2
Keur Boumi	**N6**/Souna	Ziguinchor	**S6**/Sanio	Thiénaba	**C6**/Souna	Sinthiou	**E6**/ICTP 8203
Keur Boumi	**N7**/*P.violaceum f*	Niaguis	**S7**/Souna	Dakar	**C7**/Souna	Mountôgou	**E7**/Souna
Ngaraf	**N8**/Souna	Bignona	**S8**/Sanio	Paoskoto	**C8**/Souna		
Louga	**N9**/Chibra	Diouloulou	**S9**/Sanio	Diameguene	**C9**/Souna		
Louga	**N10**/Souna	Birkama*	**S10**/Sanio	Kahone	**C10**/Souna		
Louga	**N11**/Souna	Vêlingara	**S11**/Sanio	Nioro	**C11**/Souna 3		
Barale Ndiaye	**N12**/Chibra	Saré Bassi	**S12**/Sanio	Keur Baka	**C12**/Souna		
Thiamène	**N13**/*P.violaceum f*	Tankanto	**S13**/Sanio	Sanguel	**C14**/Souna		
Dara	**N14**/Souna			Ndoffane	**C15**/Souna		
Thiélé	**N15**/Souna			Kaffrine	**C16**/Souna		
Linguère	**N16**/Souna			Diagnel	**C17**/Sanio		
				Koungueul	**C18**/Souna		
				Birkelane	**C19**/Souna		
				Baba Garage	**C20**/Souna		
				Diourbel	**C21**/Souna		
				Gossas	**C22**/Souna		
				Niakhar	**C23**/Sanio		
				Gorgui Bêwêté	**C24**/Souna		
				Doubalampor	**C25**/Souna		
				Taba	**C26**/Souna		

Kamboinse (Burkina Faso) : isolates **BF1, BF2**
* = locality in Gambia

[GAGGGTGGCGGTTCT, (Karlsson, 1994)] were used to amplify as follow: 4 minutes with 94°C, 35 cycles (1 min at 48°C, 1 min at 72°C and 1 min at 94°C), 10 min at 72°C. DNA amounts used were equal for each PCR amplification (100 ng). A volume of 8 µL of PCR products (1.25 µg/µL) was deposited on agarose gel. The electrophoretic patterns were analysed by using PAUP software (Swofford 1993). The tree was drawn with the TreeView software 1.5.2.

RESULTS

The use of M13 primer allowed differentiating thirteen main electrophoretic patterns (Fig. 2). Considering each band as a marker useful for a distance matrix, 21 polymorphic characters were identified among the different isolates: 61 from Senegal, 2 from Burkina and 1 from Gambia. The corresponding distance tree (Fig. 3) allows the identification of 5 different clades: i) clade I is represented by the isolates of Burkina Faso; ii) The clade II is formed by isolates essentially collected from the North of Senegal and are not met in any other zone; iii) the isolates on clade IIIare essentially originating from the Central and the East zones and are divided in two sub groups IIIa and IIIb; the IIIa genotypes are limited to the central zones (Baba garage, Khombole, Diourbel, Diagnel, Ndoffane, Diameguene) although all these localities are not necessarily near to each other; the IIIb genotypes were found in different areas of Senegal: mostly in central and East zones, but also 4 isolates from the North and 1 isolate from the South; Genotypes IV: this clade groups 14 isolates among which 12 were collected from the South and two were harvested in the Central region (C4 and C8) in two relatively distant zones.

By reporting these genetic data on the site of collection on the map of Senegal (Fig. 1), it appears a North-South and central East-central West gradient of distribution of genotypic variability, although these populations are not strictly genetically isolated.

DISCUSSION

In this work, we highlighted that the population of *Moesziomyces penicillariae* is constituted of sub populations genetically different. We differentiated 4 main clusters of genotype. This genetic diversity is not randomly spread in Senegal, suggesting that environmental factors support this distribution. The isolates from South and North regions of Senegal are

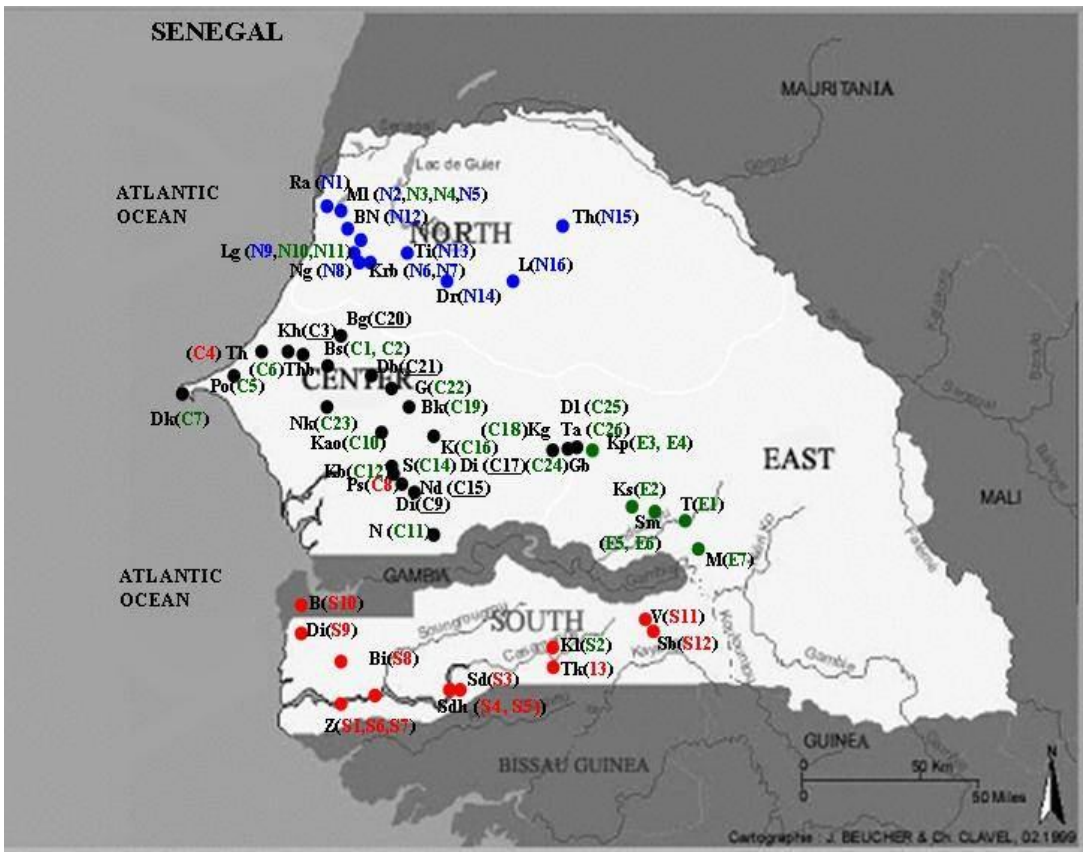

Figure (1): Distribution map of the strains collected in Senegal. Colours used for the codes of strains are function of their distribution on the radial distance tree (Figure 2): blue for clade II, black underlined for clade IIIa, green for clade IIIb, and red for cladeIV. The abbreviated names of the localities are listed in table 1.

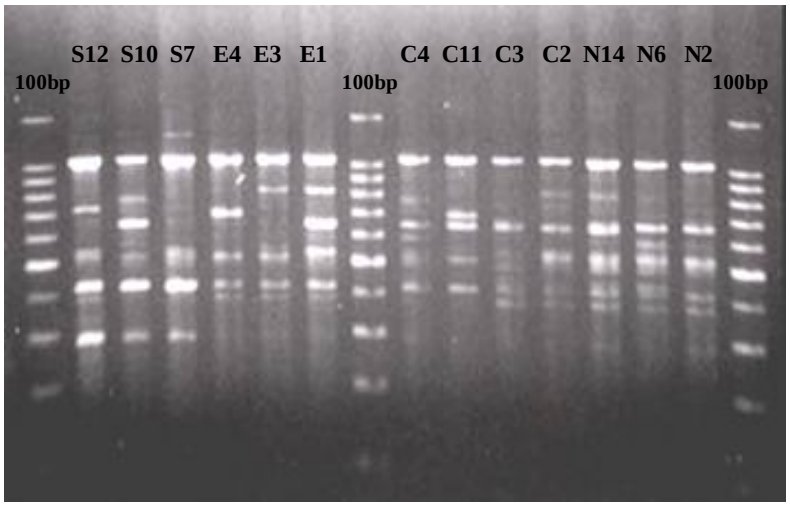

Figure (2): Electrophoretic patterns selected after amplification by M13 primer of DNA of the 61 isolates

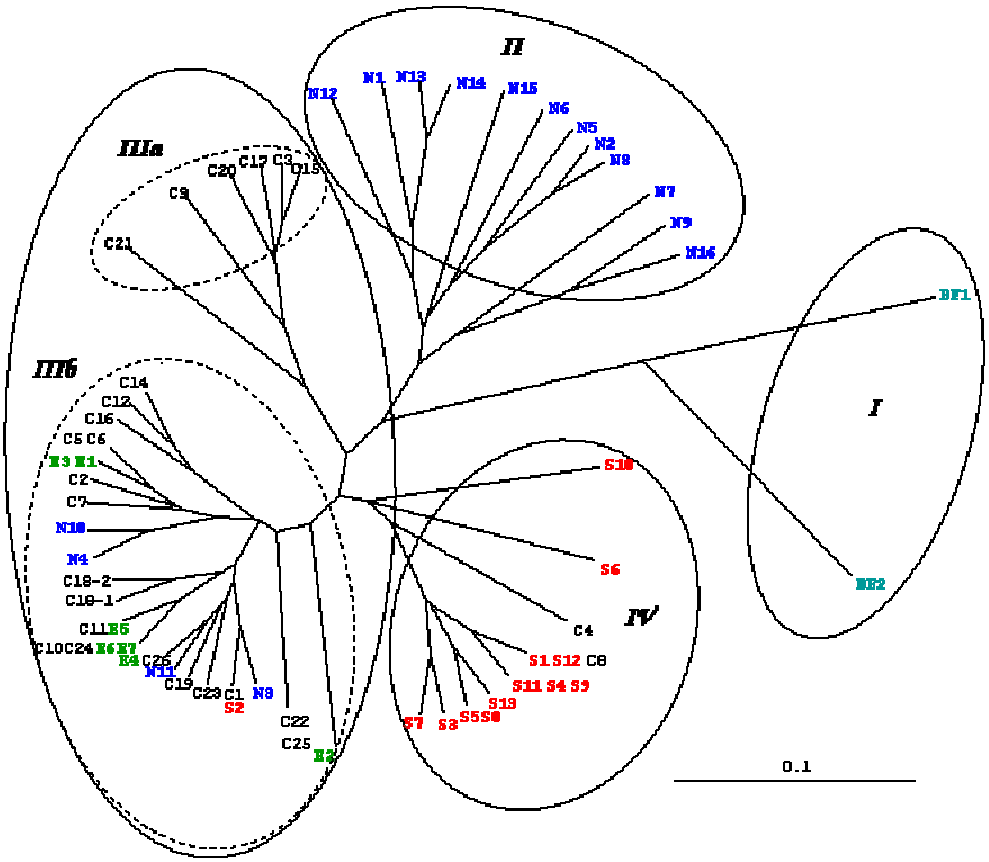

Figure (3): Radial distance tree presenting the different regroupings among isolates, obtained by UPGMA analysis from 21 discriminative characters revealed by amplification of the inter-minisatellite M13 regions. Codifications of strains (cf table 1, and Figure 1) use the initial S (South), C (Center), E (East) and N (North) for the strains collected in Senegal; BF for the strain collected in Burkina Faso. The ellipses surround genotypes joined together in a same clade.

grouped in separate clades. Although two populations were characterised in Central and Eastern zone of Senegal, the distribution of these genotypes is not strictly correlated to the geographic origin of the isolates, specifically on genotypes IIIb. In the wetter south (average of rainfall, about 45 up to 650mm), the pearl millet variety "sanio" is generally cultivated. The restrictive use of one host variety can be at the origin of the specialization of strains genetically related in the South region. In the same way, the drier climate of the North zones (average of rainfall about 0 up to 260mm) and the use of new adapted cutivars (IBV8004) could be also at the origin of the genetic homogeneity of the isolates collected in the North region. This distribution seems to be partly correlated to the climatic conditions and the host varieties adapted to these areas. Genetic mixing appears easier between the isolates of Central East and Central West zones (about 0 up to 360mm), since relative strict regrouping doesn't exist to these two geographical origins. This genetic mixing could be related to the use of seeds ("souna 3") provided by more productive zones causing new contaminations. It's interesting to point out that the presence in the North area of genotypes from clade IIIb, composed of genotypes mainly present in the Center of Senegal, was localised on sites served by railway. This observation is in accordance with the hypothesis of a dissemination related to the trade of goods and seeds, supporting the role of anthropochory in the dispersal of the smut disease. In the Central region of Senegal, although a higher genetic diversity was observed, the isolates from Dakar to Tambacounda were grouped in two sister clades (IIIa and IIIb). It must be stressed that in the central area the climatic conditions are homogenous, allowing the use of the same cultivars of Pearl millet, whereas in the North, the hot and dry climate needs appropriate varieties. These results reveal that the distribution of Pearl millet smut populations in Senegal depend on two main factors: host varieties according to climate and human activities. The incidence of smut disease on Pearl millet would then be limited by the use tolerant varieties adapted to each climate. As discussed by Thakur *et al.* (1986), Pearl millet breeding tests to obtain tolerant varieties must take in account the genetic diversity of the fungus from the different regions. Lastly, it must be point out that our data on genetic diversity indicate that the dispersal of this disease is moderate. Thus, an efficient prophylactic method to limit the incidence of this disease would be to establish a sanitary control of the seeds and to alert the farmers not to use seeds from infected inflorescences for sewing.

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