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### RESEARCH ARTICLE

#### CHARACTERIZATION OF *FUSARIUM* SPECIES ASSOCIATED WITH MANGO MALFORMATION DISEASE IN SOUTHERN SENEGAL

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#### Abstract

In Senegal mango production has long existed in traditional forms, especially in the south and center of the country. It accounts for 60% of the country's fruit production, with an estimated annual output of 150,000 tons. Despite a positive balance sheet in recent years, mango orchards are affected by numerous phytosanitary constraints like diseases and insect pests. Among the diseases, mango malformation is considered one of the most deadly for the mango tree. Since its discovery in 2009 in the south of Senegal, the disease has continued to spread in this area with incursions towards the northern part of the country. Few studies were dedicated to that pathogen in the Senegalese context. This study was carried out to help bring more light in the identity of the pathogen(s) causing this disease. Therefore 13 isolates of *Fusarium* isolates obtained from mango malformation tissues were characterized using morphological criteria and the molecular analysis. The 5.8S rDNA region of the internal transcribed spacer (ITS) was amplified using ITS1 and ITS4 primer pairs. Sequence analysis and other analytical studies showed that the malformation of mango tree in southern Senegal is related to a diversity of *Fusarium* species. The morphological and molecular analysis allowed to identify several *Fusarium* strains with a very high degree of similarity (99.08 to 99.82%) with the species in the NCBI database ranged.

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#### Introduction:-

The mango tree (*mangifera indica* L.) is considered one of the most important fruit crops in tropical regions. World production is estimated at 43 million tons according to FAO statistics in 2015. Asia is the largest producer with 76.3% of world production, followed by America with 12.3% and Africa with 11.4% (Faostat, 2015).

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Mangoes are grown in most West African states, with a production area of 540,000 km<sup>2</sup>, stretching from Senegal through western Gambia to eastern Nigeria (Ministère du commerce, 2016). Several countries in the subregion, including Senegal, are currently using it as their main export activity (CARE, 2009).

In Senegal mango production has long existed in traditional form, especially in the south and center of the country. The reduction of the main cash crops, such as groundnuts and millet, has led farmers to seek alternative income-generating activities. To this end, some producers in the south and center have seen the modernization of traditional orchards and the creation of new mango plantations for export. Since then, mango production accounts for 60% of the country's fruit production, generating an estimated annual output of 150,000 tons (Diedhiou *et al.*, 2014). The mango sector becomes the most dynamic in fruit exports in the country (Diouf, 2016). Mango exports to the European Union and the countries of the subregion increased from 300 tons in 1998 (Rey, 2011) to 16770 tons in 2015 (Diouf, 2016).

The mango-producing regions are : Dakar, Thies, Saint-Louis, Fatick, Kolda, Ziguinchor and Sedhiou (Diedhiou *et al.*, 2014). However, despite a fairly positive balance sheet in recent years, mango production is affected by numerous phytosanitary constraints, including mango malformation disease. It is probably considered as the most crucial disease of mango at present (Nafees *et al.*, 2010; Kumar *et al.*, 2011), not only because of its widespread and destructive nature, but also because of its confused etiology since its discovery despite several years of research. Economic losses have been estimated in several countries to be between 10-90% (Kumar *et al.*, 2016). The mango malformation disease manifests itself as a vegetative malformation and a floral malformation that directly reduces yield because the affected panicles become sterile (Kumar *et al.*, 2011; Freeman *et al.*, 2014a). Several abiotic and biotic stresses are associated with mango malformation disease. Following several years of continuous research, fungi of the genus *Fusarium* spp. have been most often reported as the main cause of this disease (Iqbal *et al.*, 2010; Iqbal *et al.*, 2011; Freeman *et al.*, 2014a; Kumar *et al.*, 2016). Several species of *Fusarium* have been identified worldwide. The disease was first recorded in West Africa in southern Senegal in 2009 in Tandouk in the department of Bignona (Senghor *et al.*, 2012). Subsequently it was also reported in Mboro in Thies region in 2012 (Senghor *et al.*, 2012). The disease has been progressing steadily in the South since its discovery where the incidence of the disease ranged from 20 to 91% (Dieye *et al.*, 2020). Thus, it is urgent to know all the *Fusarium* species associated with mango malformation disease in southern Senegal. The morphological characterization of *Fusarium* species is very complex despite their differences at the colony level, the forms of macroconidia, the presence or absence of microconidia, chlamydospores and the formation of secondary structures are very delicate criteria to differentiate in some cases (Leslie et Summerell, 2006). This study aims to identify the *Fusarium* species responsible for mango malformation using morphological characterization as well as molecular analysis of 13 *Fusarium* isolates obtained from samples of malformed mango sampled from southern regions of Senegal.

## Materials and methods:-

### Study site:

Samples were taken from the roads in the southern regions (Tambacounda, Kolda, Sedhiou and Ziguinchor) of Senegal (Figure 1). The climate is of the Guinean Soudano type and characterized by heavy rainfall during the rainy season.

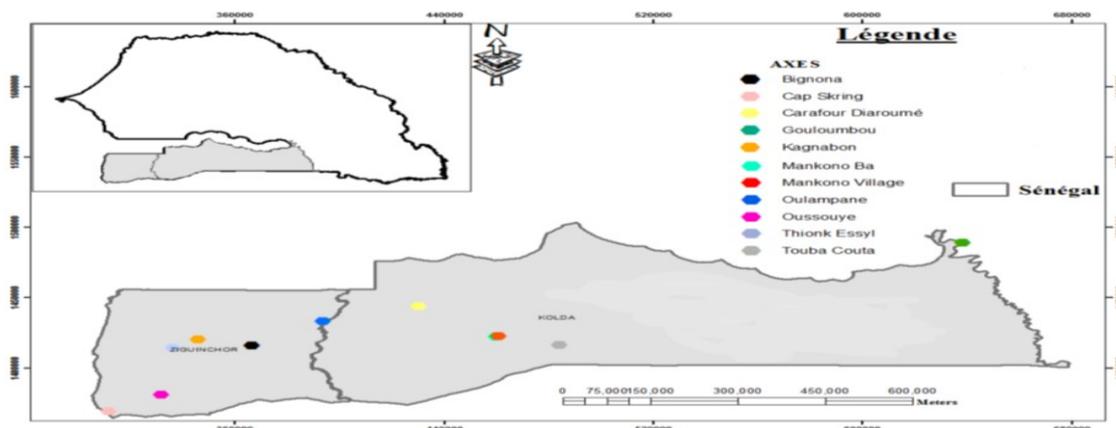


Figure 1:- Location of Sampling Sites.

**Sampling:**

Sampling was carried out every 50 ± 5 km on 30 mango trees at a rate of 10 feet chosen at random in 3 directions per locality from Velingara to Ziguinchor and Ziguinchor to Diouloulou (Figure 1).



Floral malformation (A)      Vegetative malformation (B)  
**Figure 2:-** Floral and vegetative malformations collected in Sedhiou.

**Isolation and purification:**

The samples (floral and vegetative malformations) were first disinfected with 1%. Then, using a sterile scalpel blade, explants were taken. Under the fume hood, these explants were successively immersed in 70% alcohol, rinsed with sterile distilled water, wiped with sterile absorbent paper and finally inoculated with 3 explants per Petri dish containing the PDA medium. The plates are incubated in the oven at 25°C. Few days after, some of the mycelium is transferred to other plates to obtain pure strains.

**Single-spore culture protocol:**

It was carried out using Booth's 1971 protocol to obtain genetically homogeneous fungal material (Booth, 1971). For this purpose, the isolates were first transplanted to Petri dishes containing PDA medium and allowed to grow over the entire surface of the dish for 7-10 days. A 5mm diameter mycelial disc was taken from the periphery and introduced into a tube containing 9ml sterile distilled water. After shaking, dilutions to one hundredth are made with a sterile pipette. This operation is repeated as many times as necessary until the desired dilution is reached. Then a volume of 0.1ml generally containing 2 to 5 microconidia adjusted with the Thomas cell was taken from the last 2 dilutions and spread with sterile beads on a 2% agar medium. Identification and delimitation of germinating spores was performed using a binocular magnifying glass after 48h incubation at 25°C. Fragments of 3 to 4 of these conidia are collected with their agar using a sterile loop and placed in Petri dishes containing PDA before being incubated at 25°C for one week. This operation was repeated for all the different isolates. This operation was repeated for all the different isolates.

**Study of morphological variability:**

To study the morphological variation between different *Fusarium* spp. isolates, each isolate is transferred to a Petri dish containing a PDA medium combined with chloramphenicol to avoid bacterial contamination. The inoculation was done using a 5mm diameter mycelial disc taken aseptically with a fume hood punch from a 10-day old *Fusarium* sp. culture before incubation at 25°C. The daily evolution of the mycelial diameter of the different isolates was regularly done for each replicate. The mycelial diameter was measured on the horizontal and vertical planes of the box and the average of the two measurements was calculated. This procedure was carried out until the mycelium had grown over the entire surface of the box.

**Morphological characterization:**

The macroscopic study was done based on the colour on the surface and reverse side of the boxes, the growth rate of the mycelium and the pigmentation. While the microscopic description was done by observing the shape of the microconidia, macroconidia, phialides and chlamydospores. In addition, a biometric study was carried out on 50 conidia per isolate.

**Molecular characterization:**

Molecular identification was done using the regions of the internal transcribed spacer. DNA was extracted from mycelia grown on Petri dishes containing potato dextrose agar (PDA) medium. DNA extraction of each isolate was performed using the BIOLINE Isolate II Plant DNA kit according to the kit protocol. Then a Nanodrop assay was performed prior to PCR. A PCR reaction was prepared for all 13 DNA samples according to the following protocol using ITS1 / ITS4 primers.

Reagents (MyTaq DNA polymerase kit from Bioline)	Quantity for 1 tube
2x buffer	10µl
ITS1 10µM	2µl
ITS4 10µM	2µl
DNA	120ng
H2O	QSP for Vt = 25µl

The PCR was performed from an ABI "Verity" Thermocycler according to the following program: 95°C, 2min; (95°C, 30sec; 57°C, 30 sec; 72°C, 30sec) 35x; 72°C, 3min.

PCR products were detected by migration electrophoresis on 1% agarose gel in the presence of a 1Kb molecular weight marker. The photos were visualized by the photo documentation system "G Box". The PCR products from the amplified DNA samples were sent to the sequencing laboratory for further analysis.

After sequencing and processing by BLAST, the consensus sequences were compared to sequences in the NCBI GenBank database. The identification of each isolate was defined based on the closest percentage resemblance to the Blast results. A phylogenetic tree was then constructed using MEGA 10.1.6 software (Kumar *et al.*, 2018) using the Maximum Likelihood algorithm with 1000 bootstrap replications (Felsenstein, 1985). The resulting sequences were subsequently submitted to the NCBI for accession number assignment.

**Results:-****Morphological characterization:**

A total of 13 *Fusarium* isolates were obtained following mono-spore culture and morphological variability. Morphological characters exploitation allowed a provisional identification to be made (Table 1). Thus, 5 species were associated with the 13 strains of *Fusarium* studied, i.e. *Fusarium mangiferae* (3), *Fusarium subglutinans* (5), *Fusarium oxysporum* (2), *Fusarium sterilihyphosum* (1), *Fusarium equiseti* (1) and one strain with less obvious criteria was noted as *Fusarium* sp.

**Table 1:-** Morphological characters of the 13 *Fusarium* isolates obtained from samples collected in southern Senegal

Iso lat es	S and R Colonies	Pigme ntation	Chlam ydospo res	Phiali des	Microconidia		Macroconidia				Nearer species
					Shape	Size (µm)	Shape	Ap ex	Base	Size (µm)	
2	Whitish and Creamy	Violet Absen t	Violet Absent	mono et poly	Ovoid	4,02- 13,7 9	Fusiform	pap illa	under develop ed	20,2 7- 35,8 2	<i>F.</i> <i>sterilihy phosum</i>
3	White and cream	White Absen t	White Absent	mono et poly	Oval	6,62- 13,3 7	thin, straight and little curved	cur ved	foot shape	18,4 5- 41,5 6	<i>F.</i> <i>mangife rae</i>

5	White and cream	White Absent	White Absent	monopoly	oval to obovoid	6,33-15,66	straight and a little curved	Blunt	notched	19,26-39,26	<i>F. mangiferae</i>
6	White and cream	White Absent	White Absent	monopoly	Oval	6,75-12,32	slim and straight	curved	underdeveloped	17,76-47,46	<i>F. subglutinans</i>
61	Purple White and Violet	Violet Absent	Violet Absent	monopoly	allantoid	6,49-13,83	thin and a little curved	Beak	underdeveloped	18,45-28,35	<i>F. oxysporum</i>
7	White and cream	White Absent	White Absent	monopoly	Oval	6,03-14,12	thin, medium and straight	tapere d	foot shape	21,48-33,13	<i>F. subglutinans.</i>
8	White and cream	White Absent	White Absent	monopoly	obovoid to oval	4,61-13,60	thin straight to curved	curved	foot shape	18,39-50,76	<i>F. mangiferae</i>
9	White and cream	White Present	White Present	mono	oval a obovoid	4-13,09	slim and straight	Blunt	underdeveloped	21,44-43,82	<i>F. oxysporum</i>
11	Whitish and Violet	Violet Absent	Violet Absent	mono	Ovoid	4,05-13,49	mine and fusiform	Sle n d e r	underdeveloped	19,06-30,67	<i>F. subglutinans</i>
12	Whitish and purple	Violet Absent	Violet Absent	monopoly	oval to ovoid	4,04-12,60	Fusiform	Sle n d e r	foot shape	18,02-51,98	<i>F. subglutinans</i>
17	White-orange and orange	Yellow Present	Yellow Present	monopoly	straight thin	10,98-18,89	spindle-shaped and curved	Sle n d e r	foot shape	28,48-64,98	<i>F. equiseti</i>
26	White and Cream	White Absent	White Absent	monopoly	Oval	4,82-12,62	slim and straight	Ho o k e d	underdeveloped	18,58-43,16	<i>F. mangiferae</i>
53	Light brown	beige	Absent	mono	napiform		Not observed				<i>Fusarium sp.</i>

### Molecular Characterization:

Amplification of the ITS region of the ribosomal DNA of *Fusarium* spp strains with the ITS 1 and ITS 4 primers resulted in bands of sizes between 500 and 600 base pairs.

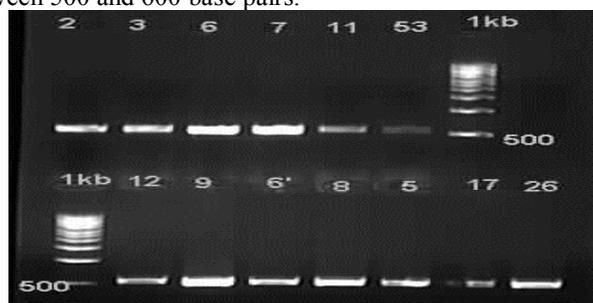


Figure 3:- DNA fragment from the ITS region of *Fusarium* strains after PCR.

The sequences obtained after sequencing from the PCR products were submitted to the NCBI reference database by BLASTn for identification of the species studied. The results obtained showed that strain 2 had 99.26% resemblance with *Fusarium* sp. ASPB1-Buralikson while strain 3 had 99.45% resemblance with *Fusarium subglutinans* and *Fusarium proliferatum* TF1. Strains 5 and 6 had 99.45% and 99.42% similarity with *Gibberella moniliformis* strain EXGF-2 and *Fusarium circinatum* isolate L-943/2013, respectively. When strain 7 showed 99.25% resemblance with *Fusarium sterilihyphosum* isolate EFA 6FSRG while strains 8 and 9 successively showed 99.62% resemblance with *Fusarium verticillioides* strain AE-FPO8 and *Fusarium circinatum*. Whereas, strain 11 showed 99.64% resemblance with *Fusarium sudanense* and *Gibberella thapsina* isolate M3790. Strains 12 and 17 showed 99.08% and 99.82% resemblance with *Gibberella moniliformis* isolate FM7 and *Fusarium equiseti*, respectively. In addition, strain 26 showed 99.44% resemblance to *Fusarium subglutinans* as well as to *Fusarium proliferatum* and *Fusarium sterilihyphosum*. And finally strain 53 showed 99.82% resemblance with *Gibberella moniliformis* STRAIN Novb1.

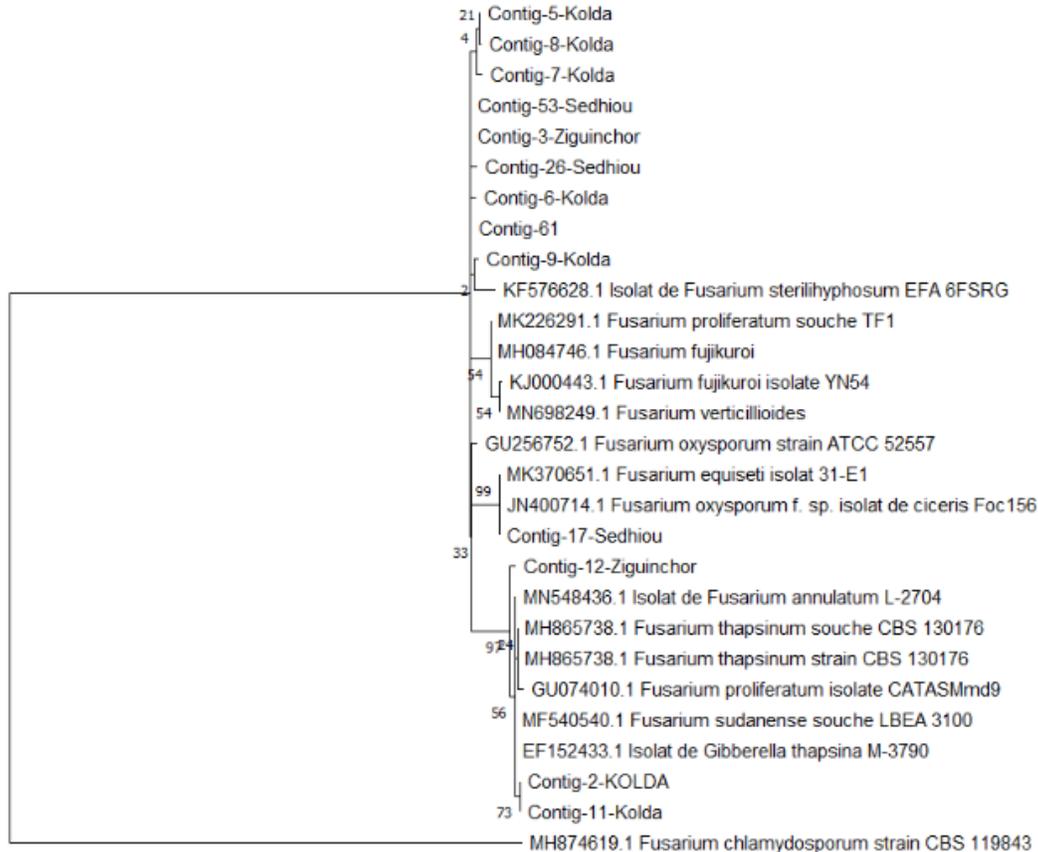
**Table 2:-**Degree of similarity between the *Fusarium spp.* isolates studied and the reference species in the NCBI database.

Strains	Number of bp after assembly	Nearby species	% d'identification
2	543	<i>Fusarium sp. ASPB1-TR-Buralikson</i>	99,26
		<i>Fusarium fujikuroi strain Bt4L</i>	99,25
		<i>Fusarium proliferatum isolate EFS12</i>	99,25
3	544	<i>Fusarium subglutinans</i>	99,45
		<i>Fusarium proliferatum TF1</i>	99,45
		<i>Fusarium sp. 3. BRO-2013</i>	99,45
5	545	<i>Fusarium fujikuroi strain Bt4L</i>	99,44
		<i>Gibberella moniliformis strain EXGF-2</i>	99,45
6	521	<i>Fusarium circinatum isolate L-943/2013</i>	99,42
7	548	<i>Fusarium sterilihyphosum isolate EFA 6FSRG</i>	99,25
8	554	<i>Fusarium verticillioides strain AE-FPO8</i>	99,62
		<i>Fusarium proliferatum strain Train TF1</i>	99,44
		<i>Fusarium fujikuroi FZ04</i>	99,44
9	543	<i>Fusarium circinatum</i>	99,62
11	560	<i>Fusarium sudanense</i>	99,64
		<i>Gibberella thapsina isolate M3790</i>	99,64
		<i>Fusarium annulatum 2704/2012</i>	99,46
12	558	<i>Gibberella moniliformis isolate FM7</i>	99,08
17	540	<i>Fusarium equiseti</i>	99,82
26	543	<i>Fusarium subglutinans</i>	99,44
		<i>Fusarium proliferatum</i>	99,44
		<i>Fusarium sterilihyphosum</i>	99,44
53	548	<i>Gibberella moniliformis STRAIN Novb1</i>	99,82

Figure 4 represents the phylogenetic tree showing the relationships between the *Fusarium spp.* isolates studied (contigs-5-Kolda, 8-kolda, 7-Kolda,53-Sedhiou, 3-Ziguinchor, 26-Sedhiou, 6-Kolda, 61-Sedhiou, 9-Kolda, 17-Sedhiou, 12-Ziguinchor, 2 and 11- Kolda) and some reference species in the NCBI database. The phylogenetic tree can be divided into two main clades. The first clade is made up of 4 sub-clades where all the isolates studied are present except 12-Ziguinchor, 2 and 11 Kolda isolates. The firstsub-clade with a bootstrap value less than 50%, formed by the isolates contig 5 Kolda, contig 8 Kolda and contig 7 Kolda are closer to KF576628 1 *Fusarium sterilihyphosum* EFA 6FSRG isolate from the NCBI database as well as the contig 53 Sedhiou, 3 Ziguinchor, 26 Sedhiou, 6 Kolda and contig 61 isolates which do not belong to any subclade. The second subclade with a bootstrap value of less than 50% formed by contig 9 Kolda and the reference strain of the NCBI *Fusarium sterilihyphosum* database. The third subclade consisting of MK226291. 1 *Fusarium proliferatum* strain TF1, MH084746 1 *Fusarium fujikuroi*, KJ000443 1 *Fusarium fujikuroi* isolates YN54 and MN698249 1 *Fusarium verticillioides* from the database. And the fourth subclade with a bootstrap value of 99% consisting of 2 reference strains from the NCBI database (MK370651. 1 *Fusarium equiseti* isolate 31-E1; JN400714. 1 *Fusarium oxysporumf sp. ciceris* Foc 156) and Contig-17 Sedhiou.

The second clade with a bootstrap value of 97% consists of 3 subclades.

A first subclade formed by Contig-12-Ziguinchor, a second subclade containing 6 reference species from the NCBI database (MN548436. 1 *Fusarium annulatum* isolate L-2704, MH865738. 1 *Fusarium thapsinum* strain CBS 130176, MH865738. 1 *Fusarium thapsinum* strain CBS 130176, GU074010. *Fusarium proliferatum* isolate CATASMd9, MF540540. 1 *Fusarium sudanense* strain LBEA 3100 and EF153433. 1 *Gibberella thapsina* isolate M-3790). And the third subclade with a bootstrap value of 73% formed by contig-2 Kolda and contig-11-Kolda which are closer to *Gibberella thapsina* M-3790. All species studied are probably related to MH874619. 1 *Fusarium chlamyosporium* strain CBS 119843.



**Figure 4:-** Phylogenetic tree showing the relationships between the *Fusarium* spp. isolates studied based on ITs gene sequence using the Maximum Likelihood algorithm with 1000 bootstrap replications.

Accession numbers of contigs-5-Kolda (N accession MN944572), 8-kolda (N accession MN944576), 7-Kolda (N accession MN944575), 53-Sedhiou (N accession MN944573), 3-Ziguinchor (N accession MN944571), 26-Sedhiou (N accession MN944570), 6-Kolda (N accession MN944674), 61-Sedhiou (N accession MN944578), 9-Kolda (N accession MN944577), 17-Sedhiou (N accession MN944568), 12-Ziguinchor (N accession MN944567), 2-Kolda (N accession MN944566) and 11-Kolda (N accession MN944569)

### Discussion:-

Morphological characterization of the strains isolated from the mango floral malformation in southern Senegal has made it possible to observe different fungal structures. These are microconidia, chains of microconidia, hyphae, macroconidia, monophialides, polyphialides and chlamyospores, typical of *Fusarium* (Leslie et Summerell, 2006) including some species associated with mango malformation disease identified by (Britz *et al.*, 2002; Senghor *et al.*, 2012). These results confirm that mango malformation disease in southern Senegal is associated with a species complex of the genus *Fusarium*. With the exception of *F. equiseti*, which belongs to the Gibbosum section, and *F. oxysporum*, all other species belong to the Gibberella section. Thus, the isolates showed cultural and microscopic similarities with the species *Fusarium mangiferae*, *Fusarium subglutinans*, *Fusarium oxysporum*, *Fusarium*

*sterilihyphosum*, *Fusarium equiseti* and an isolate which could not be assimilated to any of these species: mycelia sterilia.

It should be noted that the morphological characterization of *Fusarium* spp. is very complex. The culture criteria used allowed a provisional identification of the species. However, this identification may be influenced by a number of parameters, namely the composition of the culture medium, the purity of the culture of the isolates studied, the incubation conditions, etc. According to Leslie and Summerell, (2006), some species producing chlamydospores may be very close to those that do not produce chlamydospores as these spores are not conserved over time. In 2012, species isolated from mango malformation in southern Senegal were very close to *F. mangiferae*, *F. sterilihyphosum* and *F. tuiense* following morphological characterization. In this study, isolates 7 and 26 were also compared to *F. sterilihyphosum*.

With the exception of isolate 17, which was identified as *F. equiseti*, the other species are otherwise closely related to the Fujikuroi complex which includes *F. Fujikuroi*, *F. mangiferae*, *F. proliferatum*, *F. verticilloides*, *F. circinatum* and *F. oxysporum* fsp. *lycopersici*. All these species share more than 90% similarity in their genome. The difference may lie in the species and isolate specificity on the host plant in vivo and in vitro in the expression of specific genes responsible for the synthesis of molecules that may play a role in pathogenesis (Niehaus *et al.*, 2016).

Comparison of the sequences of *Fusarium* spp. isolates isolated in this study with the sequences of reference species in the NCBI database showed degrees of similarity ranging from 99.08 to 99.82% with several *Fusarium* species. The results obtained did not completely correspond to those of the morphological characterization except for isolates 17 and 26. Isolate 17 was morphologically associated with *F. equiseti* and molecular characterization showed 99.82% similarity with the same species. Isolate 26 morphologically resembled *F. subglutinans* and its comparison in the database showed 99.44% similarity with *F. subglutinans*, *F. sterilihyphosum* and *F. proliferatum*. These two species have already been associated with mango malformation disease around the world according to several studies (Steenkamp *et al.*, 2000; Britz *et al.*, 2002; Zhan *et al.*, 2012; Freeman *et al.*, 2014b) This result is probably due to the fact that the genus *Fusarium* is a genus that is evolutionarily recent and therefore unstable, hence the importance of the number of species and the variations between species on the same plant and from one plant to another in this genus.

The abundance of one species relative to another can also vary from one region to another depending on environmental conditions, including anthropogenic activity, presence or absence of sexual forms, stage of infection, and host plant receptivity conditions (Leslie *et al.*, 2011).

With regard to the results of the morphological characterization and molecular study, it should be noted that, despite the high degree of similarity between the *Fusarium* spp. isolates studied and the reference species in the NCBI database, morphological differences were also noted. In addition, most of the species selected as a result of morphological characterization did not coincide with those obtained during sequence comparison in the NCBI database, which shows the limitations of the methods used when further comparison is carried out. Even for molecular analyses there are limitations and there is also the problem of the repeatability of the results, especially since the method is essentially based on the search for similarity with already identified species.

It was interesting to see the relationships between the *Fusarium* spp. isolates studied and some reference species in the NCBI database in so far as mango malformation disease is associated with several species of the *Gibberella fujikuroi* complex such as *F. subglutinans* (O'Donnell *et al.*, 1998); *F. mangiferae* (Britz *et al.*, 2002), *F. sterilihyphosum* (Britz *et al.*, 2002), *F. tuiense* (Lima *et al.*, 2012; Crespo *et al.*, 2016) among others. Thus, the resulting phylogenetic tree revealed that isolates could show a high degree of similarity with a reference species in the NCBI database and be distant on the tree. This was found for most of the strains studied with the exception of isolate 17.

In the end, even with the results of the molecular characterization, it did not seem possible to identify exactly which *Fusarium* species corresponded to the isolates studied, although it did allow for greater accuracy. This could be explained by the fact that the primers used were not very specific and do not always allow to distinguish very close species due to the variability of ITS zones. But also that these species are very phylogenetically close. Thus, it would be important to be more precise in the molecular approach by using specific primers based on genes well represented in the database such as the  $\beta$ -tubulin and elongation factor -1 $\alpha$ .

**Conclusion:-**

Thus, from this study on the characterization of isolates associated with mango malformation disease in Senegal, it appears that the malformation of mango in Senegal is due to species of the genus *Fusarium* which are phylogenetically very similar. One morphologically closer to *Fusarium mangiferae* is probably the most implicated in the disease while the others are thought to play a complementary role in the infection process of mangoes, as is the case for other plant crops attacked by *Fusarium*.

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**Bibliographical References:-**

1. **Booth, C. 1971.** The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England.
2. **Britz, H., E. T. Steenkamp, T. A. Coutinho, B. D. Wingfield, W. F. Marasas and M. J. Wingfield 2002.** "Two new species of *Fusarium* section *Liseola* associated with mango malformation." *Mycologia* **94**(4): 722-730.
3. **CARE, S. 2009.** « Analyse filière de la mangue en Casamance naturelle, région de Ziguinchor », P. PASCO.: 51p.
4. **Crespo, M., F. Cazorla, A. De Vicente, E. Arrebola, J. Torés, M. Maymon, S. Freeman, T. Aoki and K. O'Donnell 2016.** "Analysis of genetic diversity of *Fusarium tuiense*, the main causal agent of mango malformation disease in southern Spain." *Plant disease* **100**(2): 276-286.
5. **Diedhiou, P. M., S. A. G. Diop, N. Mbaye, I. Diedhiou, Y. Diallo, S. Djiba, R. Faye and P. Samb 2014.** "Mango rotting in southern Senegal, a big phytosanitary challenge." *International Journal of Biosciences (IJB)* **5**(5): 183-188.
6. **Dieye, C., N. MBAYE and P. M. Diédhiou 2020.** "DISTRIBUTION OF THE MANGO MALFORMATION DISEASE IN SOUTHERN SENEGAL." *International Journal of Science, Environment and Technology* **9**: 362 – 372.
7. **Diouf, M. J. M. 2016.** Bilan de la campagne 2015 d'exportation mangue au Sénégal. . Dakar, ASEPEX. **1**: 5p.
8. **Faostat, F. 2015.** "Agriculture organization of the United Nations, 2011." *FAO*. Retrieved from <http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/S>. Accessed 20.
9. **Felsenstein, J. 1985.** "Confidence limits on phylogenies: An approach using the bootstrap. ." *Evolution* **39**: 783-791.
10. **Freeman, S., M. Maymon, A. Biton, A. Levin and D. Shtienberg 2014a.** "Management of mango malformation disease based on a novel strategy of timing of fungicide applications combined with sanitation." *Crop Protection* **61**: 84-91.
11. **Freeman, S., D. Shtienberg, M. Maymon, A. G. Levin and R. C. Ploetz 2014b.** "New insights into mango malformation disease epidemiology lead to a new integrated management strategy for subtropical environments." *Plant disease* **98**(11): 1456-1466.
12. **Iqbal, Z., N. Akhtar, M. U. Ghazanfar, S. M. Shehzad, S. Ahmad, M. Asif, M. Yasin and M. A. Pervez 2011.** "Management of mango malformation through physical alteration and chemical spray." *African Journal of Agricultural Research* **6**(7): 1897-1901.
13. **Iqbal, Z. M. A., B. A. Pervez, Saleem, A. Salman, A. D. Altaf and A. Saleem 2010.** "Potential of *Fusarium mangiferae* as an etiological agent of mango malformation." *Pak. J. Bot.* **42**(1): 409-415.
14. **Kumar, P., M. Kamle, A. K. Misra, A. O'Donovan, M. Pagano and D. R. Modi 2016.** "Identification and characterization of *Fusarium mangiferae* as pathogen of mango malformation in India." *Brazilian Archives of Biology and Technology* **59**: 1-9.
15. **Kumar, P., A. K. Misra and D. R. Modi 2011.** "Current status of mango malformation in India." *Asian Journal of Plant Sciences* **10**(1): 1-23
16. **Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura 2018.** "MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms." *Molecular Biology and Evolution* **35**: 1547-1549.
17. **Leslie, J. and B. Summerell 2011.** "In search of new *Fusarium* species." *Plant Breeding and Seed Science* **63**(1): 94-101.
18. **Leslie, J. F. and B. A. Summerell 2006.** The *Fusarium* laboratory manual. Blackwell, 2121 State Avenue, Ames, Iowa 50014, USA.

19. Lima, C. S., L. H. Pfenning, S. S. Costa, L. M. Abreu and J. F. Leslie 2012. "Fusarium tupiense sp. nov., a member of the Gibberella fujikuroi complex that causes mango malformation in Brazil." *Mycologia* **104**(6): 1408-1419.
20. Ministère du commerce, d. s. i. e. d. P. M. E. 2016. La Semaine de la Mangue du Sénégal autour du thème : La Mangue! Délicieux trésor de l'Afrique de l'Ouest, Note conceptuelle et Agenda général.: 15.
21. Nafees, M., R. Anwar, M. Jameel, M. N. Aslam, S. Ahmad, F. Akhtar and N. Memon 2010. "Flushing pattern of mango (*Mangifera indica* L.) cultivars in response to pruning of panicles and its effect on carry over effect of floral malformation." *Pak. J. Agri. Sci* **47**(1): 13-18.
22. Niehaus, E.-M., M. M. y, R. H. P. y, A. S. Daren W. Brown, Yifat Idan, L. Oren-Young, C. M. Sieber, O. Novak, Ales Pencik, Danuš e Tarkowska, Kristy'na Hromadova, Stanley Freeman, Marcel Maymon, Meirav Elazar, Sahar A. Youssef, El Said M. El-Shabrawy, Abdel Baset A. Shalaby, Petra Houterman, Nelson L. Brock, Immo Burkhardt, Elena A. Tsavkelova, J. S. Dickschat, Petr Galuszka, Ulrich Gu' Idener and Bettina Tudzynski 2016. "Comparative "Omics" of the Fusarium fujikuroi SpeciesComplex Highlights Differences in Genetic Potential and Metabolite Synthesis." *Genome Biol. Evol.* **8**(11): 3574–3599.
23. O'Donnell, K., E. Cigelnik and H. I. Nirenberg 1998. "Molecular systematics and phylogeography of the Gibberella fujikuroi species complex." *Mycologia* **90**(3): 465-493.
24. Rey, J. Y. 2011. La production fruitière sénégalaise. Thies.3p
25. Senghor, A. L., K. Sharma, P. L. Kumar and R. Bandyopadhyay 2012. "First report of mango malformation disease caused by *Fusarium tupiense* in Senegal." *Plant disease* **96**(10): 1582-1582.
26. Steenkamp, E., H. Britz, T. Coutinho, B. Wingfield, W. Marasas and M. Wingfield 2000. "Molecular characterization of *Fusarium subglutinans* associated with mango malformation." *Molecular Plant Pathology* **1**(3): 187-193.
27. Zhan, R.-L., S.-J. Yang, F. Liu, Y.-L. Zhao, J.-M. Chang and Y.-B. He 2012. "First report of *Fusarium mangiferae* causing mango malformation in China." *Plant disease* **96**(5): 762-762.