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ASSESSMENT OF TOLERANCE TO FUSARIUM WILT OF SOME TRADITIONAL ACCESSIONS OF OIL PALM (*ELAEIS GUINEENSIS* JACQ.) COLLECTED IN MAN, WEST OF COTE D'IVOIRE

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ABSTRACT

The oil palm (*Elaeis guineensis* Jacq.) genetic improvement is based on a recurrent reciprocal selection scheme, involving two groups of populations whose production components are complementary. Group A is essentially coming from Asia, while group B includes African oil palm populations. In order to increase genetic variability and to enrich the agronomic qualities of this group B, a survey was carried out in Western Côte d'Ivoire. Twelve traditional genotypes were collected in the Man area. They are known to produce a rather fluid palm oil but their behavior against *Fusarium* wilt was unknown. Therefore, these traditional populations were subjected to the *Fusarium* wilt tolerance test. One hundred and sixty (160) two-months-old seedlings of the traditional accessions were distributed in 8 completely randomized blocks and inoculated by *Fusarium oxysporum* f. sp. *elaedis*. The appearance of external symptoms on inoculated plantlets was observed over the duration of 5 months, and internal symptoms remarked after plantlets dissection. Index of the *Fusarium* wilt susceptibility of each progeny was determined. A third of traditional genotypes tested (Dompleu Kp 03, Gbangbegouiné Doua 01, Gbangbegouiné Kla 01 and Gbatonguin Yod 02) proved to be highly tolerant to the wilt disease. Four traditional accessions (Bogouiné Sad 02, Dompleu Kp 01, Dimgouin Zoh 02 and Blolé Dio 05) showed low tolerance, while the last four traditional genotypes (Blolé Oul 03, Koutongouiné Iba 02, Blolé Dio 02 and Dompleu Dou 03) were sensitive to the wilt disease.

Keywords: Fusarium disease, oil palm, tolerance, traditional genotypes.

INTRODUCTION

The genetic improvement of oil palm is relatively recent. It is based on a scheme of recurrent reciprocal selection modelled on the selection model developed by Comstock *et al.*, (1949) for maize. This selection scheme has been adopted by the research institute for oil and oleaginous (IRHO) since 1957 (Meunier and Gascon, 1972). The recurrent reciprocal selection involves two principal groups of oil palm populations with complementary production components (Durand-Gasselin *et al.*, 2000). The Group A is characterized by palms with a small number of large bunches (Deli) and Group B, the La Mé and Yangambi populations (West Africa), includes palms

with a large number of small bunches.

Oil palm's improvement by the recurrent reciprocal selection is composed of successive cycles. Each cycle includes progeny tests (Group A × Group B crosses) to identify the best parents, which were then recombined within each group (within-group recombination) to make up the improved populations or basic populations for the next cycle (Noumouha *et al.*, 2016). More than half a century of selection has brought about an estimated genetic progress of about 60% (Durand-Gasselin *et al.*, 2009). Nevertheless, during the manipulations, a reduction of the gene pool of the populations used occurs (Cao, 1995). It is therefore essential to maintain greater

genetic variability of basic populations in order to maintain a sustainable improvement program. To this end, an investigation was carried out in 2014 in the Western Area of Côte d'Ivoire, especially in the region of Man. Traditional oil palm populations of this area are known to produce fluid oil with undeniable organoleptic qualities. So, they have interesting agronomic traits to enrich the collection available at IRHO / La Mé.

However, the performance of these traditional genotypes in the face of the wilt was entirely unknown. Indeed, vascular wilt is the most devastating pathology of oil palm plantation in Africa (Renard and Franqueville, 1989). Caused by the fungus *Fusarium oxysporum* f. sp. *elaeidis* (Foe), this disease induces estimated losses of 50 or even 80% of palm trees in plantations (Allou *et al.*, 2009; Diabaté *et al.*, 2014).

As a vascular disease, the only effective means of fighting this wilt is the selection of tolerant material (Renard *et al.*, 1972). Initiated by Prendergast (1963) and improved by Renard *et al.* (1972), this selection is based on the inoculation of oil palm plantlets with Foe. This one allows early detection of the tolerant material.

Thus, this study's aim was to evaluate the performance of some traditional oil palm genotypes in the face of vascular wilt caused by *F. oxysporum* f. sp. *elaeidis* (FOE).

MATERIALS AND METHODS

Study site: In Côte d'Ivoire, the genetic improvement of oil palm is running at National Center for Agronomic Research (CNRA, formerly represented by IRHO). Seedlings from these traditional genotypes have been prepared for the selection service of La Mé. And the tolerance test was carried out at the experimental and production station Robert Michaux (Dabou) (Figure 1). This research station is located about 60 km west of Abidjan on the road linking the cities of Dabou and Grand-Lahou (5° 20'N; 4°20'W) (Traoré and Péné, 2016). This station is located in a savannah zone, which is an unusual vegetation inside the subequatorial forest of the south of Côte d'Ivoire (Caliman, 1990). The climate is characterized by four seasons, two seasons of rains (March - July and October - November) and two dry seasons (December - February and August - September). For this last decade, the average annual rainfall was about 1700 mm, the average monthly temperature was about 26°C and relative humidity was over 85%. Soils are poor in fine elements like all soils of southern parts of Côte d'Ivoire (Perraud, 1971). The soils of savannah of Dabou are essentially sandy-clayey with a more clayey tendency

in areas of steeper slope (Traore *et al.*, 2010).



Figure 1. Map of Côte d'Ivoire presenting the different study areas.

Plant material: The 12 oil palm accessions used in the study were collected in the region of Man, West of Côte d'Ivoire (Figure 1). They were coded using the name of the locality from which they were taken, followed by a grower code and the number of the tree visited i.e Blolé Dio 02, Blolé Dio 05, Blolé Oul 03, Bogouiné Sad 02, Dimgouin 02, Dompleu Dou 03, Dompleu Kp 01, Dompleu Kp 03, Gbangbegouine Doua 01, Gbangbegouine Kla 01, Gbatonguin Yod 02 and Koutongouiné Iba 02. All accessions were composed of traditional genotypes.

Fungal material: The pathogenic strain of *F. oxysporum* f. sp. *elaeidis* (FOE) used to inoculate seedlings was the Mono 179. This fungal strain was isolated from the tissues of diseased oil palm trees and then cultured monosporelly. Mono 179 is usually used to set up the inoculation tests in the phytopathology laboratory of research station of Dabou.

Experimental design: The experimental design comprised of 8 completely randomized blocks. A block constituted of 12 basic plots, and each basic plot had 20 seedlings of each traditional genotype. A total of 160 seedlings belonging to

each oil palm accession was used for the test.

Inoculation: The preparation of the inoculum with the fungal strain Mono 179 was carried out according to the methods described by (Gbongué *et al.*, 2012) and (Diabate *et al.*, 2013), as follows:

The collar of Two months old palm seedlings was cleared to expose the roots, and roots were scraped with a sterile stick to create lesions. Wounded roots were rinsed with distilled water and 20 ml of inoculum containing an average of 1.7×10^7 spores of the FOE was introduced. Following successful spore inoculation, roots of plantlets were carefully covered with potting soil and monitored for the development of disease symptoms.

Disease evaluation: The symptoms recording has begun the first month after inoculation. Leaves symptoms and the stunted plantlets were recorded. For 05 months, all plantlets had been monthly observed and counted according to their health status. So, each month, a tested genotype has been characterized by a percent of infected plantlets (presence of external symptoms). After 05 months, the pseudo-bulb of each plantlet had been cut open so that the browning of tissues which indicates the presence of the parasite in the plantlet. And then, each genotype has been characterized by a percent of infected plantlets (presence of internal symptoms).

Definition of the Index: The performance of one tested genotype in the face of the vascular wilt is defined in relation to the mean percentage of Wilt-stricken plantlets (external and internal symptoms) in the whole test. Thus, to each traditional accession corresponds a value obtained by the following formula:

$$I = \frac{\text{Percent of wilt - Infected plantlets of progeny} \times 100}{\text{Percent of wilt - infected plants in all progenies tested}}$$

In these conditions, the progenies with low indices are the most resistant to Wilt. It can be accepted that an acceptable

estimate of the threshold for a tolerant genotype lies at the mean index 100. Hence, the progenies which have an Index of more than 100 are considered as being sensitive.

Data analysis: External symptoms were evaluated according to a kinetic evolution and by the rate of diseased plants of each oil palm's accession. And, internal symptoms were only estimated according to the rate of diseased plants of each oil palm's accession. The rate of diseased plants is calculated from the formula:

$$RWP = \frac{NWP}{TNP} \times 100$$

Where;

RDP: Rate of Wilt-infected plantlets

NDP: Number of Wilt-infected plantlets

TNP: Total number of plantlets

External symptoms evolution was presented as curves and the expression of internal symptoms in relation to traditional genotypes was presented by histograms drawn by the Microsoft spreadsheet (Excel). The mean rate of Wilt-infected plantlets had been calculated by Statistica 7.1 Stat Soft. The mean rate of Wilt-infected plantlets was compared with an ANOVA 1. In case of significant differences ($p=0,05$), the mean rates have been segregated by test of Newmann-Keuls.

RESULTS

Description of the symptoms

External Symptoms: From the first month after the inoculation of oil palm seedlings with FOE some of the seedlings already had the appearance of being infected. They had a stunted appearance with a much more pronounced leaf yellowing generally on the last leaf (Figure 2A). Some of the diseased plantlets had commonly shown triangular-shaped perforations on the main vein of the last leaf (Figure 2B).



Figure 2. External symptoms observed on oil palm seedlings. A - Two seedlings of the same genotype, which have the

same age, 1: a normal seedling and 2: a stunted seedling because of the pathology. B - An infected plantlet that presents a partial yellowing of the sheet and characteristic triangular perforations.

All the traditional varieties tested had reacted to the presence of the pathogenic fungus and the aforementioned external symptoms amplified over time thus increasing the rate of infected seedlings. In most traditional progenies, the rate of Wilt-infected

plantlets increased until the third and the fourth month before moving towards stabilization (Figure 3). The dissection of infected plantlets had revealed some brown spots in the tissues of pseudo-bulb, especially at the base (Figure 4).

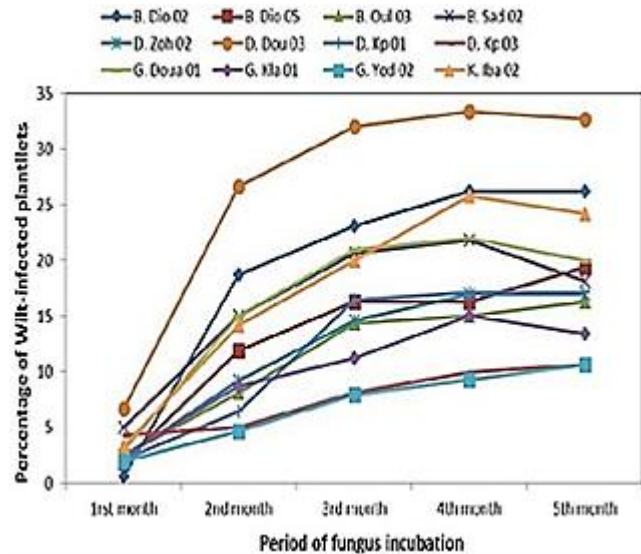
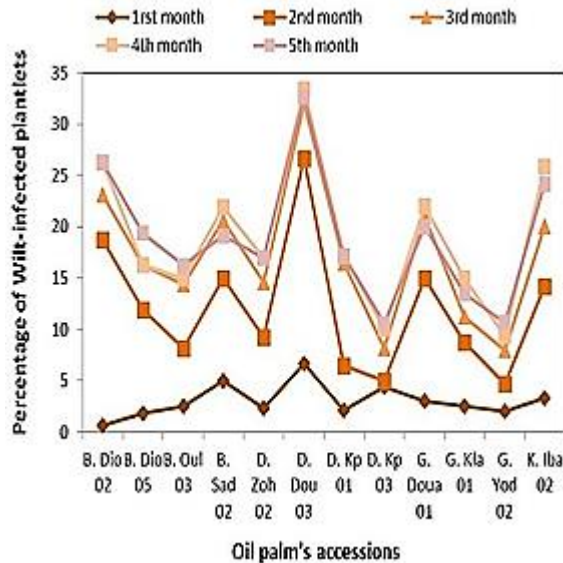


Figure 3. Evolution of the external symptoms of *Fusarium* wilt. A - Evolution of symptoms richness over time. B - Evolution of symptoms degree according to the genotypes tested.

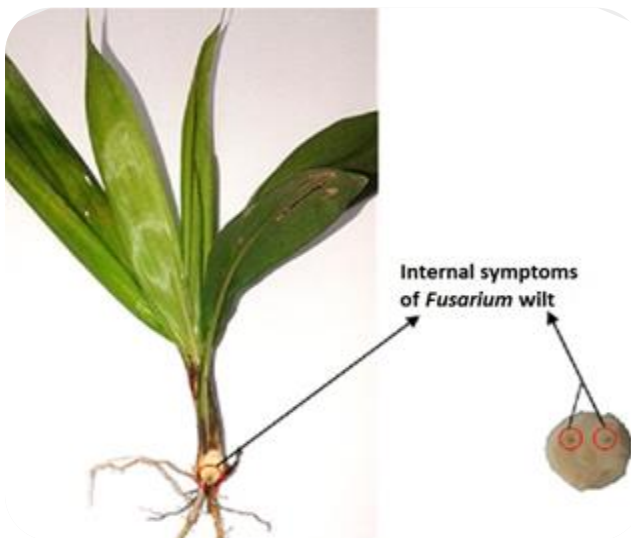


Figure 4. Internal symptoms of *Fusarium* Wilt. Left - Longitudinal section of the pseudobulb showing the brown spot. Right - Transversal section of the pseudobulb showing the marks of O2 brownish vessels.

All the traditional genotypes tested have shown internal

symptoms with a rate of infected plantlets, which was lower than 50 %. The highest percentage (40.78 %) was observed in the oil palm population of Dompleu Dou 03 and the lowest percentage (15.00 %) was recorded in the population of Gbatongouiné Yod 02 (Figure 5).

Susceptibility to FOE of the traditional oil palm populations: The calculation of the index made it possible to classify the traditional oil palm accessions in order of increasing sensitivity (Table 1).

DISCUSSION

This work represents the first series of tests on some genotypes whose performance in the face of the vascular Wilt was entirely unknown. After inoculating the oil palm seedlings with the pathogenic fungus, the leaflets of some plantlets exhibited curbing and yellowing, attesting that these ones were infected by *Fusarium oxysporum* f. sp. *elaedis* (Renard and Ravisé, 1986; Renard and Franqueville, 1989). Besides, some plantlets were stunted, and triangular perforations have appeared on the main vein of one of the last leaves. The presence and the progressive appearance of these symptoms are the consequence of plantlets

colonization by the pathogenic fungus (Agris, 2005). In fact, the fungus living in the soil enters through the roots and moves through the vessels of plants. These ones become

brownish like the tissues observed after the pseudo bulb's dissection. Thus, the sap can no longer get into the infected vessels that are obstructed by FOE (Laville, 1962).

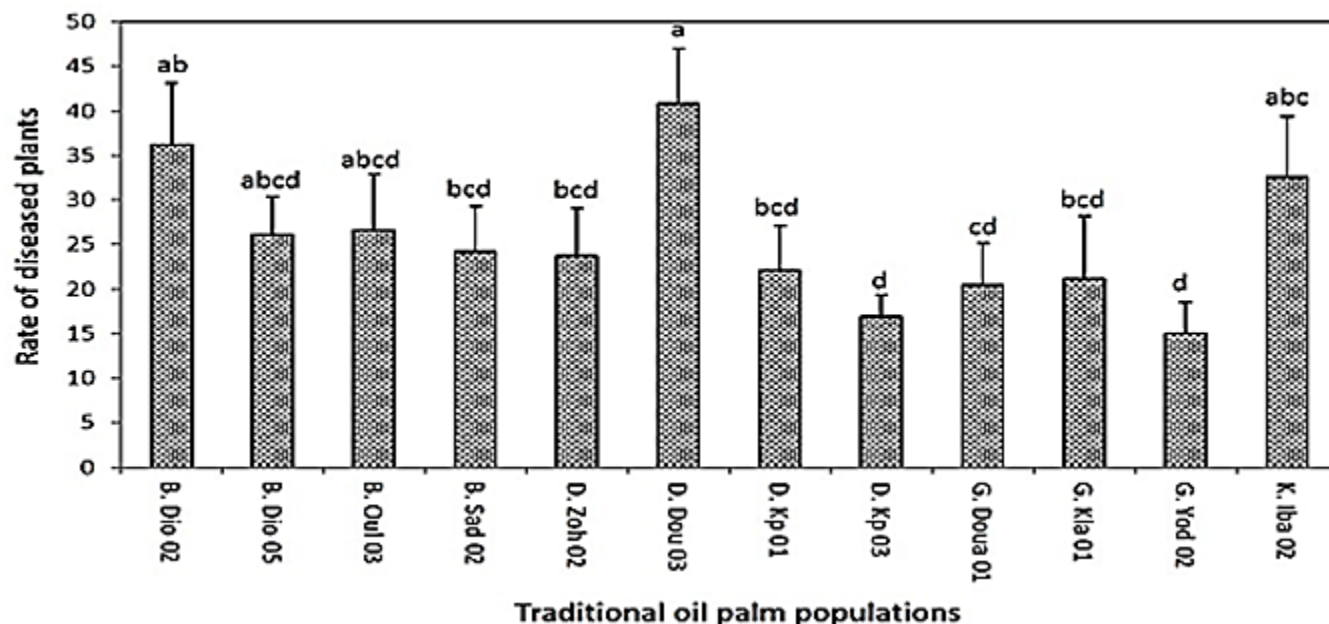


Figure 5. The rate of infected plantlets, which are presenting internal symptoms.

Table 1. Classification of traditional genotypes.

Traditional genotypes tested	Wilt Index obtained
Gbatonguin Yod 02	61
Dompleu Kp 03	64
Gbangbegouiné Kla 01	78
Gbangbegouiné Doua 01	87
Bogouiné Sad 02	91
Dompleu Kp 01	93
Dimgouin Zoh 02	96
Blolé Dio 05	98
Blolé Oul 03	100
Koutongouiné Iba 02	132
Blolé Dio 02	137
Dompleu Dou 03	160

After the classification, 03 trends have emerged from this ranking:

- i. Firstly, the traditional accessions; Blolé Oul 03, Koutongouiné Iba 02, Blolé Dio 02 and Dompleu Dou 03 had got respective index values of 100, 132, 137 and 160. So, these genotypes were sensitive to *Fusarium* wilt.
- ii. Secondly, the traditional oil palm progenies with Index values comprised between 90 and 100; Bogouiné Sad 02 (91), Dompleu Kp 01 (93), Dimgouin Zoh 02 (96) and Blolé Dio 05 (98) were considered to express a relatively low tolerance.
- iii. Finally, the genotypes that have demonstrated tolerance for *Fusarium* wilt belong to the oil palm accessions of Gbatonguiné Yod 02, Dompleu Kp 03, Gbangbegouiné Kla 01 and Gbangbegouiné Doua 01. They had got respective index values of 61, 64, 78 and 87.

Moreover, 05 months after the inoculation of the plantlets with the dissection of some pseudo bulbs revealed brown tissues corresponding to the zones infected by the pathogenic fungus (Renard and Franqueville, 1989). In fact, the brown tissues result from the obstruction of the vascular system of the plantlets due to the presence of fungi *Fusarium oxysporum*.

All genotypes that were tested in this study have presented external symptoms of *Fusarium* wilt from one month after inoculation. However, the previous studies estimated that generally, external symptoms appear after the first month of FOE incubation in plantlets (Gbongué *et al.*, 2012; Kablan *et al.*, 2016). This difference in the time of onset of external symptoms would be due to genotype. In fact, the traditional oil palm genotypes used in this study had not yet been the subject of a genetic study, unlike those used in the kinetic studies of expression of *Fusarium* wilt of oil palm.

On the other hand, with most traditional genotypes, the rate of diseased plantlets increased during the second and third months after inoculation and then stabilized from the fourth month. However, with genotypes of Gbangbegouine Doua 01 and Gbangbegouine Kla 01, a slight decrease in the rate of infection was rather observed.

Variations observed in the rate of infected plantlets could be explained by the induction of defence mechanisms following a pathogenic aggression (Bozarth and Ross, 1964; Ross, 1966; Dean and Kuć, 1987). Indeed, in presence of abiotic aggression, plants activate their defense mechanisms in different ways. But, generally, defense mechanisms are based on chemical reactions, which produce some chemical substances such as phenolic compounds (Diabate *et al.*, 2009).

The first phase of defense mechanisms that is the detection of the pathogen by plantlets, could explain the fact that symptoms appear just a month after inoculation (Guo and Stotz, 2007). Certainly, recognition of the pathogen, which had to take place in a shorter time interval, led to an amplification of the fungus action in plant tissues (Holub and Cooper, 2004; Diabaté *et al.*, 2014). Thus, an accentuation of the expression of the disease could occur. But, once the pathogenic fungus has been detected, defense responses are induced and new pathogenesis-related proteins are produced (Clériveret *et al.*, 1996). Also, the speed of detection of the pathogen associated with the defenses developed by the plant would determine the nature of its reaction in the

presence of the pathology (Benhamou, 2009).

Traditional populations tested in this trial can be grouped into three (03) susceptibility groups to *Fusarium oxysporum* f. sp. *elaeidis*. The first group consisting of genotypes Dompleu Kp 03, Gbangbegouiné Doua 01, Gbangbegouiné Kla 01 and Gbatonguin Yod 02 has a high level of tolerance. In the second group, the genotypes Bogouine Sad 02, Dompleu Kp 01, Dimgouin Zoh 02 and Blolé Dio 05 were weakly tolerant to Foe. The third group, containing genotypes Bole oul 03, Koutongouiné Iba 02, Blolé Dio 02 and Dompleu Dou 03 was susceptible to *Fusarium oxysporum* f. sp. *elaeidis*. In this last group, the presence of the fungus would be detected very slowly and the developed defenses would not be sufficient to inhibit the action of the pathogen (Jones and Dangl, 2006; Ramirez-Suero, 2009; Turner, 2009). In tolerant genotypes, defenses systems would be based on their ability to reduce the toxins produced by the pathogenic organism (Afifi *et al.*, 2003).

This study showed some traditional genotypes that are tolerant to the vascular wilt. These genotypes are most interesting for the oil palm breeding program.

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