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Original Research Article

Vascular Wilt Disease Tolerance Status of Some Oil Palm (*Elaeis guineensis* Jacq.) Progenies in Relation to Local Strains of *Fusarium oxysporum* f. sp. *elaeidis* in Cameroon

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Abstract

Oil palm is the highest oil yielding crop in the world. However, its yield in Africa is reduced by vascular wilt caused by *Fusarium oxysporum*. This study was aimed at re-assessing the vascular wilt tolerance status of some palm progenies used for commercial seed production at Dibamba with local *Fusarium* strains. Ten seedling categories were inoculated with *Fusarium* in a completely randomized block nursery design. Results showed a highly significant difference of morphological parameters between the seed categories except for stem diameter. The tolerant/susceptible status of the seed categories was confirmed. C2501IIFX showed highest disease tolerance and slow growth rate. Two new highly tolerant categories (C1001II and C1901II) were revealed. Disease incidence was less intense on leaves, with the least incidence on both leaves and pseudo-bulbs observed on C2501IIFX seedlings. Disease severity was more pronounced on leaves. The least severity on pseudo-bulbs was observed on C2501IIFX (41%). Considering pseudo-bulb symptoms to be more reliable for oil palm *Fusarium* wilt diagnosis, C2501IIFX and C2501II were respectively the most tolerant and most susceptible seed categories. These results which need further confirmation are important for farmers and could be used by breeders for further oil palm improvement.

Keywords

Disease incidence
Disease tolerance
Fusarium oxysporum
Oil palm
Severity
Vascular wilt

Introduction

World demand for fats and oils is continuously increasing, thereby enhancing the need to extend the areas planted to oil crops or the need to improve on oil yields. Oil palm (*Elaeis guineensis* Jacq.) which is the highest oil yielding crop in the world (Rival and Levang, 2014) offers the best opportunity to address this situation. The oil palm originated from Africa in the Gulf of Guinea and belongs to the *Arecaceae* family (Hartley, 1988; Corley and Tinker, 2003). Several studies have been undertaken to improve on the field performance of this crop or to widen the genetic variability and enrich agronomic qualities of its populations (Noumouha et al., 2014). However, one major problem with oil palm cultivation is yield losses due to diseases. The crop is susceptible to several diseases of which vascular wilt caused by *Fusarium oxysporum* f. sp. *elaeidis* (*Foe*) is the gravest in Africa (de Franqueville and Renard, 1990; Cochard et al., 2005; Noumouha et al., 2014). The vascular wilt disease can cause up to 70% mortality (de Franqueville and Renard, 1990; Cochard et al., 2005). In Southeast Asia, *Ganoderma* has been known to cause up to 80% mortality of oil palm, while in Latin America, bud rot disease causes up to 100% mortality (de Franqueville et al., 2001; Cochard et al., 2005).

Vascular wilt disease of the oil palm was first reported by Wardlaw in Zaire (Wardlaw, 1946; Renard et al., 1972; Renard and Ravise, 1986; Renard and de Franqueville, 1989). The causal agent, *Foe* is a fungus which is specific to oil palm. Under the most favorable conditions for disease development, up to 50 % of the palm trees may be affected. It is therefore important to always make a good choice of planting site for replanting (Renard and Quillec, 1983). The damage caused on oil palm by the disease depends on the genetic origin of the planting material, the previous crop of the planting area and cropping techniques (Renard and de Franqueville, 1989). The disease can cause severe damage on plantations with an average of 1% annual losses (Renard and de Franqueville, 1989). Since the parasite is found both in the soil and in the plant, and with the need of applying fungicide on large areas, it is difficult to fight against the disease (Renard and Ravise, 1986). Several control approaches have been proposed (Ntsomboh et al., 2012), the best being to take preventive measures through selection of tolerant planting material (Renard and de Franqueville, 1989). Replanting in affected areas require the use of planting material whose tolerance to the disease has been proved by tests using the pathogen at the pre-nursery stage and confirmed by its performance in the field (Renard et al., 1972; Renard et al., 1980; Renard and Meunier, 1983; Renard and de Franqueville, 1989).

In fact, the role of pathogen selection on plant improvement depends on the extent of genetic variation for resistance traits and their covariance with host as well as on three specific criteria. First, pathogen infection must affect host fitness; second, heritable variation in resistance/tolerance traits must occur among individuals; and third, the heritable resistance/tolerance trait must covary with the fitness of the plant host (Kover and Schaal, 2002). Based on these concepts, the existence of oil palm crosses which are tolerant to *Fusarium* wilt has been put to evidence (Prendergast, 1963; Renard et al., 1972). It is on this basis that a method developed to assess the performance of oil palm by early screening (Prendergast, 1963; Renard et al., 1972), helped to define sources of tolerance and to produce commercial hybrids displaying the trait (Renard et al., 1980; Cochard et al., 2005).

The above technique was later adapted to the development of a method for large scale evaluation of the performance of oil palm planting materials at the nursery and the pre-nursery stages (Renard et al., 1972; de Franqueville, 1984; de Franqueville and Renard, 1990). With this technique, *Fusarium* wilt assessment is routinely carried out in pre-nursery and nursery wilt tests and under natural infection by several fungal strains. In such an assessment, the incidence of wilt in a cross is recorded as the proportion of wilted palms. The performance of each cross with respect to *Fusarium* wilt is defined by an index (I) whose value is in inverse proportion to the tolerance of the progeny under consideration (Meunier et al, 1979; Renard et al, 1980). To each parent is attributed the mean of the crosses in which it is involved (Renard et al., 1972). An index less than 100 indicates the parent’s ability to transmit a certain degree of tolerance to its progeny, while a value greater than 100 entails susceptibility; the lower the “I” rating, the higher the tolerance of the cross under consideration.

An “I” rating of 100 corresponds to the mean of the percentages observed for all the crosses in a trial. The index “Ig” of a progeny represents the ratio between the percentage of palms of that progeny affected by vascular wilt and the mean of the percentages of affected palms of all the progenies of the trial. The value Ig indicates a parent's combining ability, that is, the parents ability to transmit to its progeny either a certain degree of tolerance if the value is less than 100, or susceptibility if the Ig value is greater than 100 (Diabate et al., 2010; Noumouha et al., 2014). Based on results of nursery wilt tests, the progenies used for the production of *Fusarium* wilt tolerant seeds are classified according to their I ratings as follows: I<90 for high tolerance; 90<I<100 for moderate tolerance; 100<I<120 for susceptible progenies; and I>120 for highly susceptible progenies.

A breeding program for the production of *Fusarium* wilt tolerant palms has been established in the Reciprocal Recurrent Selection (RRS) scheme used for oil palm breeding and improvement by former IRHO network research stations (Renard et al., 1972; Meunier and Gascon, 1972). The RRS scheme which uses two groups of populations with complementary production components is comprised of several successive breeding cycles (Meunier and Gascon, 1972). The Group A is characterized by palms with a small number of large bunches (Deli) and Group B, the La Me, includes palms with a large number of small bunches. Each cycle includes progeny tests (Group A × Group B crosses) to identify the best parents, which are then recombined within each group to make up the improved populations or basic populations for the next cycle (Noumouha et al., 2014). The implementation of this breeding strategy in research stations has made it possible to perform a progression of 60 % on the oil yield in over 50 years of selection (Durand-Gasselin et al., 2009; Noumouha et al., 2014). The genetic improvement and seed production at La Dibamba is based on the RRS scheme. La Dibamba whose research programme and seed production potentialities have been reviewed by Ngando-Ebongue et al. (2013), is part of the French IRHO/CIRAD network alongside other research stations in Africa such as La Mé in Ivory Coast or Pobè in Benin.

Earlier research work done with the RRS scheme in the R. Michaux experimental plantation in Ivory Coast yielded results which led to the definition of categories of hybrids to be retained or not in zones affected by vascular wilt (Renard and Meunier, 1983; de Franqueville and Renard, 1990). These results which are being implemented in research stations of the IRHO network were obtained with the use of *Foe* strains of particular origins with particular characteristics. Furthermore, a study undertaken in Cameroon revealed variability in the virulence of *Foe* strains from different sources (Tengoua and Bakoume, 2008). It is possible that the virulence of *Foe* strains used in Ivory Coast to establish seed production programmes differ from that of *Foe* strains from Cameroon. These observations prompted the necessity of re-evaluating the material being used presently for seed production in Cameroon with local *Fusarium* strains of Cameroonian origin. The main objective of this study was therefore to confirm the tolerance/susceptibility status of the progenies of some ten commercial seed categories produced by IRAD La Dibamba through screening with local *Fusarium oxysporum* f. sp. *elaeidis* strains from Cameroon. Our results could help to confirm and select more tolerant palm genotypes for commercial seed production.

Materials and methods

Study site

The seeds used for this study were produced at the specialized Centre for oil palm research of La Dibamba in the Littoral Region of Cameroon. La Dibamba is located at 3°54'62" latitude N and 9°5'77" longitude E. It is at 55m above sea level. Average rainfall in the area is 2500 mm/year, while sunshine is 1400 h/year, and average temperature is 27.50°C. Inoculum preparation and the nursery trial were done at the Ekona Regional Research Centre of the Institute of Agricultural Research for Development (IRAD). This locality is found in the Fako Division of the South West Region situated between 3°54'22" and 6°29'52" latitude north and between 8°30'58" and 10°6'45" longitude East.

Seed production

Ten categories of commercial oil palm seeds (Table 4) were produced through controlled pollination at IRAD La Dibamba and pre-germinated following the dry heat technique. They included three tolerant progenies (C1001IIF, C2301IIF and C2501IIFX) and seven susceptible progenies (C1001II, C2301II, C2501II, C2001II, C2101II, C1501II, and C1901II) to which were attributed treatment codes for convenience as presented in Table 1. All these commercial seeds originated from Deli x La Mé crosses (Table 1).

Field isolation of *Fusarium oxysporum* strains

The pathogen used for field trial was *Fusarium oxysporum* f. sp. *elaeidis* (*Foe*). The *Fusarium* samples were earlier isolated from the rachis of infected palm trees in the CDC oil palm plantations in Limbe and conserved in the laboratory through reseeding on culture medium. Isolation procedure included field cutting of the rachis of infected palms; thorough cleaning of the part concerned with alcohol and use of a sharp clean knife to remove the pericarp in the laboratory; flaming of the bench top, cutting and sample extraction materials (pincers, scalpels) each time with alcohol and by flaming. This was followed by extracting the infected area as deep as possible and fragmenting into small pieces while flaming the scalpel each time; placing of ten pieces in each Petri dish containing culture medium; incubating for three days at 25 °C in a hood; and re-seeding the fungus that develop around the fragments of rachis on new culture medium immediately it appears in order to avoid contamination. During fungal isolation, the date, plot, tree number, culture medium and manipulator’s identification were recorded respectively in a note book, on a data collection sheet, on test tubes and on the Petri dishes used (Tengoua, 1993; Tengoua and Bakoume, 2008).

Table 1. Seed categories and experimental treatment codes used in this study.

Seed Category	Treatment code	Key to abbreviations	
C1001 IIF	T1F	TF	Tolerant to *Fusarium*
C2301 IIF	T2F	N	Normal
C2501 IIFX	T3F	T1F	Treatment N° 1 of tolerant seeds
C1001 II	T1N	T2F	Treatment N° 2 of tolerant seeds
C2301 II	T2N	T1N	Treatment N° 1 of normal seeds
C2501 II	T3N	T2N	Treatment N° 2 of normal seeds
C2001 II	T4N	T2F1	First repetition of T2F
C2101 II	T5N	T1N2	2nd repetition of T1N
C1501 II	T6N	T1FO/T1N0	Controls without inoculum
C1901 II	T7N		

Inoculum preparation

Preparation of *Foe* inoculum was done in the La Dibamba phytopathology laboratory base at IRAD Ekona. Two strains of *Foe* earlier characterized as being aggressive (Tengoua and Bakoume, 2008) were used with Armstrong *Fusarium* culture medium. In the procedure (Tengoua, 1993), *Fusarium* strains were purified in an inclined test tube. Then a small fragment was deposited in a test tube containing distilled water that had been sterilized thrice with autoclave at 120°C for 45 min.

Part of the conidial suspension obtained was collected with a Pasteur pipette and spread in a Petri dish containing NASH medium. After spore germination, a fragment was collected and placed on mycelium medium (MM) in a test tube (Ntsomboh Ntsefong et al., 2015). Then a fragment of this medium containing the fungus was cut and introduced in 75 mL of sterile Armstrong medium in a conical flask. This was agitated every 10 min for four days to enhance multiplication of *Fusarium*. Then 2 mL of the solution obtained were put in 100 mL of sterile Armstrong medium in roux bottles which were inclined on a surface sterilized with alcohol and flaming. For rapid multiplication of *Fusarium*, this was agitated every 10 min for 8 days. At the end of this step, the solution obtained was mixed and ground for 10 sec in the laboratory blender (Tengoua, 1993).

Inoculation of seedlings and data collection

The nursery trial in a completely randomized block design with four repetitions (Renard et al., 1972) was also done at IRAD Ekona (Fig. 1). In the nursery, seedlings were inoculated with the *Fusarium* inoculum previously prepared. After dilution in 4 L of sterile water, one roux bottle of the inoculum was used to inoculate 200 seedlings at the rate of 20 mL per seedling. Inoculation was done 1½ months after the pre-germinated seeds were sown in the prenursery. The steps in the inoculation process were according to the procedure used by Renard et al. (1972). After 4 ½ months, leaf symptoms of wilt were recorded as an indication (stunting of first leaflet, general unhealthy appearance of seedling). A small thread was attached on the leaflet on which all leaf parameters were noted.

After 12 months, the pseudo-bulbs of seedlings were cut open so that the browning of vessels which indicates the presence of the parasite in the plant could be appreciated accurately, and independently of any visual appraisal of the leaf symptoms (Fig. 2). Diseased seedlings were appreciated by visual rating of symptoms (Bock et al., 2010; Sanjay and Shrikant, 2011) according to the disease severity scale developed by Horsfall and Heuberger (Sanjay and Shrikant, 2011). Data on seedling morphological parameters was recorded. The disease incidence and severity were calculated with the formulae used by Nehal et al. (2013) as follows:

$$\text{Disease Incidence} = \frac{Nm}{Nt} \text{ x } 100$$

Where Nm = number of seedlings infected in the trial plot; Nt = total Number of seedlings (healthy + sick) in the trial plot.

$$\text{Disease Severity} = \frac{\sum(a \text{ x } b)}{n} \text{ x } 100$$

Where Σ (a x b) = sum of products of the number of the infected seedlings (a) corresponding to the degree of infection (b); n = number of infected seedlings.

Finally, a tolerance index (I) was attributed to each seed category (Renard et al., 1972; Noumouha et al., 2014) on the basis of computed results and according to the following formula:

$$\text{Tolerance index (I)} = \frac{\%\ wilt\ of\ infected\ plants\ of\ progeny\ A}{\%\ wilt\ of\ infected\ plants\ in\ all\ progenies} x\ 100$$

Fig. 1: Completely randomized block design layout of the trial in the nursery.

Fig. 2: Freshly dissected diseased seedling of category C1501II (T6N1) compared with a healthy seedling (left) [Note visibly wilted leaves and browning of pseudo-bulb].

Statistical analyses

Analysis of variance (ANOVA) with Fisher's LSD was used to analyze the data for morphological parameters using SPSS version 17 for Windows. The significantly different means were separated by the least significant difference calculated at the threshold of 5%. Bar charts were used to compare seed germination rate with *Fusarium* wilt disease tolerance, Disease Incidence and Disease Severity on leaves and pseudo-bulbs of oil palm seedlings.

Results and discussion

Plant diseases can be quantified in several different ways such as intensity, prevalence, incidence and severity (Bock et al., 2010) or by remote sensing or by weighing diseased vs. healthy portions of an actual leaf or photograph to ascertain percent severity (Tucker and Chakraborty, 1997; Nita et al., 2003; Bock et al., 2010). Quantifying disease on plants by measuring symptoms generally falls under the broad definition of "remote sensing" defined as obtaining information about an object without having direct physical contact with it (de Jong et al., 2006 cit. Bock et al., 2010). Thus, both visual estimation of disease and using cameras or other imaging technologies to measure disease can be considered as remote sensing (Nutter, 1990; Bock et al., 2010). The approach used in this study was visual estimation of the disease by appreciating the degree of infection from external and internal symptoms (Fig. 2, 3 and 4).

Fig. 3: Tolerant progenies of Category C2301IIF (A: without Fusarium inoculums) and C1001IIF (B: with inoculum) seedlings showing no leaf symptoms.

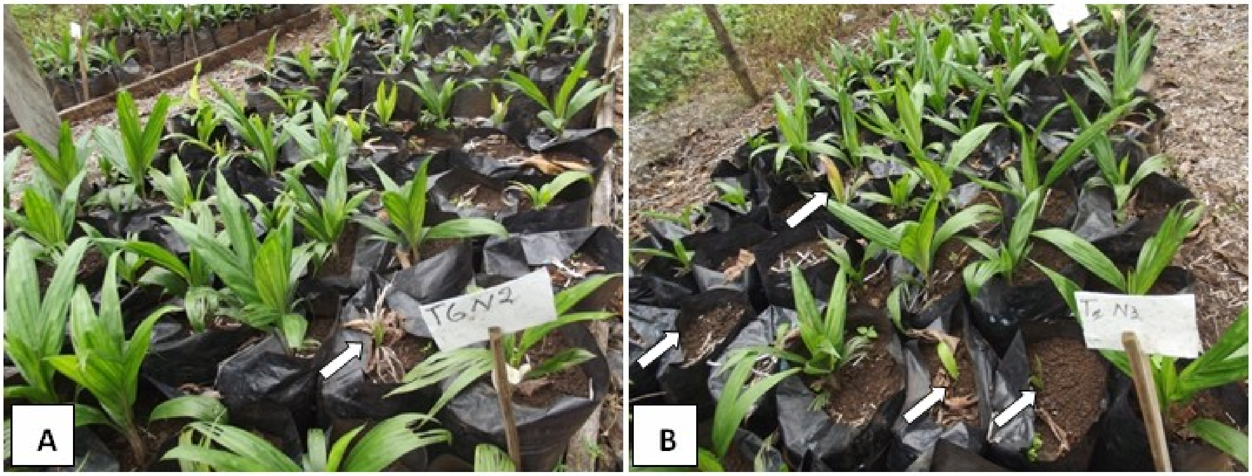

Fig. 4: Susceptible progenies (A: C1501 II and B: C1001 II) with *Fusarium* inoculums showing infected (arrows) and dead (empty sachets) seedlings.

Genotype effect on morphological parameters of seedlings

Results obtained after ANOVA of genotype effect on morphological parameters of oil palm seedlings in the nursery showed a highly significant difference of the morphological parameters (plant height, seedling stem diameter, leaflet length, width and surface area and leaf number) between the various seed categories except for stem diameter. It was also observed that there is a highly significant effect of genotype on all morphological parameters of oil palm seedlings except for the diameter where the difference is not highly significant (Table 2).

Table 2. Analysis of variance of genotype effect on morphological parameters of seedlings.

Variables	Source of variation	Sum of squares	Degree of freedom	Mean squares	F	p
Plant height	Between seed categories	1475.35	9	163.927	4.765	.00**
Seedling diameter	Between seed categories	3.92	9	.435	1.894	.05*
Leaflet length	Between seed categories	700.77	9	77.864	5.741	.00**
Leaflet width	Between seed categories	22.49	9	2.499	5.251	.00**
Leaflet number	Between seed categories	64.43	9	7.159	3.715	.00**
Leaflet surface area	Between seed categories	6801.56	9	755.729	4.997	.00**

*: Significant difference at (0.05); **: Highly Significant difference at (0.01)

Table 3. *Fusarium* wilt disease tolerance indices for ten seed categories produced at IRAD Dibamba.

Seed categories	Tolerance index (I)	Index reference	Status
C2501IIFX	23.39	I < 90	High tolerance (HT)
C1001II	52.63	I < 90	High tolerance (HT)
C1001IIF	70.18	I < 90	High tolerance (HT)
C1901II	81.87	I < 90	High tolerance (HT)
C2301IIF	99.42	90 < I < 100	Moderate tolerance (MT)
C2001II	111.11	100 < I < 120	Susceptible progenies (Sus)
C2501II	116.96	100 < I < 120	Susceptible progenies (Sus)
C2101II	128.65	I > 120	Highly susceptible progenies (Hsus)
C2301II	140.35	I > 120	Highly susceptible progenies (Hsus)
C1501II	175.44	I > 120	Highly susceptible progenies (Hsus)

Fusarium wilt disease index of seed categories

Table 3 shows the values obtained for oil palm vascular wilt index with respect to seed progenies tested in this study. References for tolerance of the oil palm to the disease are also included: I<90 for high tolerance; 90<I<100 for moderate tolerance; 100<I<120 for susceptible progenies; and I>120 for highly susceptible progenies (Ngando-Ebongue et al., 2013). The results show that in addition to the three seed categories earlier known to be tolerant to vascular wilt (C2501IIFX, C1001IIF, and C2301IIF) among the La Dibamba progenies, two other categories (C1001II with I = 52.63 and C1901II with I = 81.87) were revealed by this study to be highly tolerant. The high tolerance of C1001II could be due to the fact that it was produced from the female progeny LM 18801 known to exhibit high tolerance. Further evaluation of these two categories is required to confirm their tolerant status in order for them to be eventually considered for distribution to farmers. Category C2501IIFX also recorded the least germination rate during seed processing for this trial followed by category C2501II (Fig. 5). A comparison of disease tolerance index with the germination rate of the various seed categories used in this study reveals that this C2501IIFX with the least germination rate exhibits high disease tolerance (Fig. 5).

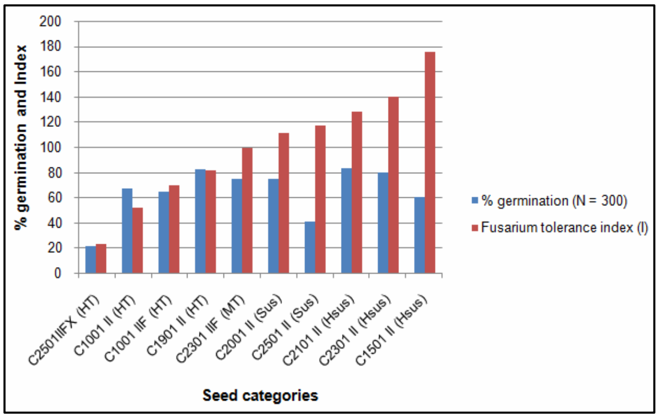

Fig. 5: Comparison of seed germination rate with *Fusarium* wilt disease tolerance index.

Table 4. Progenies used in controlled pollination for production of seeds used in this trial.

Seed category	Origin	Parentage	Female progeny	Male progeny
C2501IIFX	DA5D x DA3D	LM3257D Self	LM 13533	LM 19029
C2501 II	DA5D x DA3D	LM3038D x LM3034D	LM 17685	LM 18978
C1501 II	LM404D Self	LM3442D x LM3258D	LM 17115	LM 18978
C1001 IIF	DA 115D Self	LM2531D Self	LM 19016	LM 19029
C1001 II	DA 115D Self	LM3394D x LM3005D	LM 18801	LM 18106
C2301 IIF	LM269D x DA115D	LM5155D x LM5100D	LM 18745	LM 19029
C2001 II	LM404D x DA10D	LM2946D x LM3311D	LM 17164	LM 18978
C2301 II	LM269D x DA115D	LM5216D x LM5100D	LM 18744	LM 18978
C1901 II	DA 115D Self	LM2356D x LM2357D	LM 19171	LM 18978
C2101 II	DA10D x DA3D	LM2750D x LM2749D	LM 17163	LM 18978

Vascular wilt disease incidence

Disease incidence is the proportion (or percent) of plants (or plant units, leaves, branches, etc.) diseased out of a total number assessed (Hughes et al., 1996; Nutter et al., 1991; Madden et al., 2007; Bock et al., 2010). Mean disease incidence was obtained for each seed category during the trial (Hughes et al., 1996). Fig. 6 shows variation of disease incidence with respect to seed categories for leaves and pseudo-bulbs of ten seed categories produced at IRAD CEREPAH of La Dibamba. From results obtained in this study (Fig. 6), vascular wilt disease incidence is less intense on the leaves than in pseudo-bulbs of oil palm seedlings. This confirms the fact that the plant could be infected (in the vascular system) by the pathogen at the early stage without expressing visible symptoms. Moreover, leaf symptoms observed on some seedlings of the tolerant categories later disappeared confirming their tolerant status.

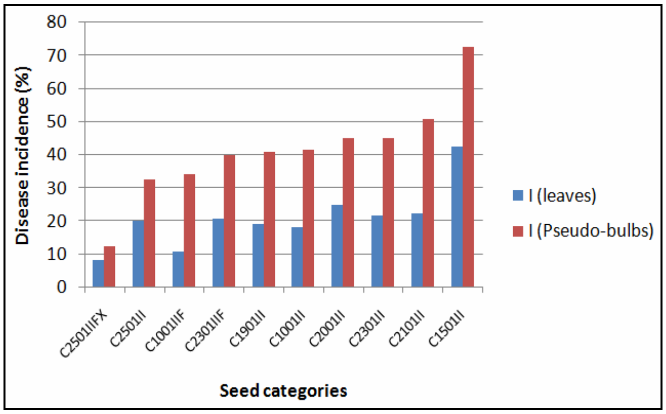

Fig. 6: Disease Incidence (I) on leaves and pseudo-bulbs of oil palm seedlings in the nursery.

The highest disease incidences on both leaves and pseudo-bulbs were recorded by category C1501II while the least was observed on seedlings of category C2501IIFX (Fig. 6). These observations are obvious, confirming their previously known status as susceptible and tolerant categories respectively. Apart from C2501IIFX whose disease incidence is higher with respect to its tolerance index, progenies of all the other seed categories showed low disease incidence for high tolerance indices. The same category C2501IIFX was observed to exhibit slow growth rate in the nursery trial. This is a very important trait exploited in oil palm genetic improvement and therefore deserves attention and further evaluation in adult palms of this category.

Disease severity

Estimates of disease severity are important for predicting yield loss in food crops, monitoring and forecasting epidemics, for assessing crop germplasm for disease resistance, and for understanding fundamental biological processes (Lindow, 1983; Bock et al., 2010). Disease severity is defined as the relative or absolute area of the sampling unit (e.g. leaf) showing symptoms of a disease. It is most often expressed as a percentage or proportion (Bock et al., 2010).

The disease severity of the plant leaves is measured by the lesion area and leaf area ratio (Bock et al., 2010; Sanjay and Shrikant, 2011). Several techniques are used to estimate disease severity on infected plants (Sanjay and Shrikant, 2011; Sungkur et al., 2009; Shen et al., 2008; William et al., 1998; Jain and Rastogi, 1998; Jennifer et al., 2004; Wijekoon et al., 2008). Of these techniques, the three major or commonly used for disease severity measurement are Visual Rating, Image Analysis and Hyper spectral Imaging (Bock et al., 2010; Sanjay and Shrikant, 2011). We chose visual rating for severity estimation in this study.

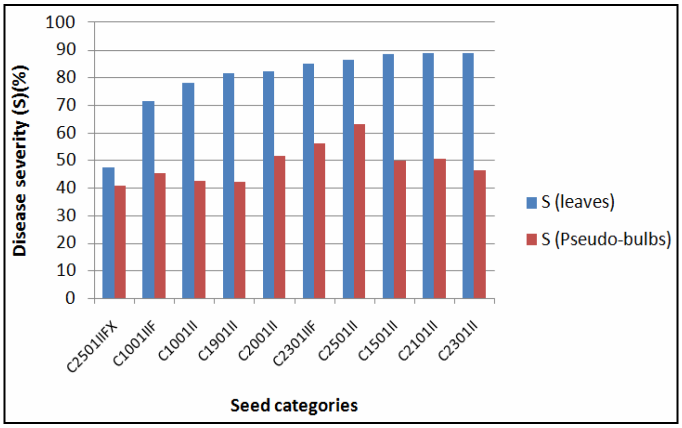

Fig. 7: Disease Severity (S) on leaves and pseudo-bulbs of oil palm seedlings in the nursery.

Results of the present study show that *Fusarium* wilt disease severity is more pronounced on leaves than in the pseudo-bulbs (Fig. 7). The highest disease severity on leaves (89%) was observed in categories C2301II, C1501II, and C2101II seedlings while the least was observed in C2501IIFX category (48%). The least severity was observed on seedlings of C2501IIFX category both on leaves and in pseudo-bulbs. Moreover, the highest disease severity on pseudo-bulbs was observed in category C2501II seedlings (63%) while the least was observed in C2501IIFX category (41%). According to these results, and considering the fact that pseudo-bulb symptoms are more reliable for oil palm *Fusarium* wilt disease diagnosis, C2501IIFX and C2501II are respectively the most tolerant and most susceptible commercial seed categories currently produced by IRAD CEREPAH.

Apart from C2301IIF (tolerant), disease severity above 80% was observed only on leaves of susceptible seed categories in the increasing order C1901II, C2001II, C2501II, C1501II, C2101II, and C2301II (Fig. 7). More still, C2301IIF (S of pseudo-bulb = 56%) is the only seed category among the tolerant progenies with disease severity in pseudo-bulbs above 50%. The pseudo-bulb disease severity values for the other two tolerant categories, C2501IIFX and C1001IIF were 41% and 45% respectively. Overall, disease severity was more pronounced on leaves than in the pseudo-bulbs (Fig. 7) with the least severity on pseudo-bulbs observed on C2501IIFX (41%).

This study has confirmed the known status of the various seed categories assessed and also revealed that some progenies which are being considered to be susceptible to the disease could be tolerant to local *Fusarium* strains. In particular, the new sources of wilt tolerance identified in this study could be completed with more sources, further evaluated and characterized molecularly (Kanchana et al., 2015) for possible introduction into the seed production programme or used to enhance progress in the next breeding cycles. These progenies are therefore a source of vascular wilt tolerance and their exploitation could make it possible to diversify sources of vascular wilt tolerance among populations used in La Dibamba breeding programme, while reducing vascular wilt incidence in Cameroon through improvement in the RRS breeding scheme. This study is useful especially for farmers to whom IRAD CEREPAH supplies the seeds for the establishment of their commercial oil palm plantations.

Conclusion

Vascular wilt disease incidence on the oil palm caused by *Fusarium oxysporum* f. sp. *elaeidis* depends more on the genetic origin of the oil palm planting materials used to establish plantations. Oil palm production could therefore be significantly improved if the losses due to diseases are reduced through the development of tolerant planting materials in breeding programmes. On the basis of the tolerance indices of the ten seed categories assessed in this study, four were highly tolerant, one showed moderate tolerance, two were susceptible progenies while three were highly susceptible progenies. Considering pseudo-bulb symptoms to be more reliable for oil palm *Fusarium* wilt diagnosis, C2501IIFX and C2501II were respectively identified as the most tolerant and most susceptible seed categories assessed. It was also observed from this study that category C2501IIF known to be tolerant seems to have slow growth rate. These two traits (tolerance and slow growth rate) could be complemented by breeding strategies. It will be important to confirm these results with field assessment of these seed categories in infected areas in Cameroon.

Results obtained are important for farmers and could be useful to breeders and crop protectionists for oil palm improvement. They could help to confirm and select more tolerant palm genotypes for commercial seed production. There is need for further assessment of local *Fusarium* strains in the laboratory and their virulence on oil palm seedlings in the nursery in order to valorize these results and improve on oil palm production through efficient vascular wilt disease management.

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References

Bock, C.H., Poole, G.H., Parker, P.E., and Gottwald, T.R., 2010. Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. Crit. Reviews Plant Sci. 29 (2), 59-107. DOI: 10.1080/07352681003617285

Cochard, B., Philippe, A., Tristan, D.G., 2005. Oil palm genetic improvement and sustainable development. OCL. 12 (2), 141-147.

Corley, R.H.V., Tinker, B., 2003. The oil palm 4th edition. Oxford: Blackwell Science LTD: 562p.

de Franqueville, Renard, J.L., 1990. Improvement of oil palm vascular wilt tolerance. Results and development of the disease at the R. Michaux plantation. Oleagineux 45: 399-405.

de Franqueville, H., 1984. Vascular wilt of the oil palm: relationship between nursery and field resistance. Oléagineux 39, 513-518.

de Franqueville, H., Asmady, H., Jacquemard, J.C., Hayun, Z., Durand-Gasselin, T., 2001. Indications on sources of oil palm (*Elaeis guineensis* Jacq.*)* genetic resistance and susceptibility to *Ganoderma sp*, the cause of basal stem rot. In: Proc. 2001 Int. Plam Oil Congr. *Agriculture*. Malaysian Palm Oil Board, Kuala Lumpur: 420-31.

de Jong, S.M., Van de Meer, F.D., 2006. Remote Sensing Image Analysis: Including the Spatial Domain. Bookseries on Remote Sensing Digital Image Processing Vol.5. Kluwer Academic Publishers, Dordrecht. ISBN: 1-4020-2559-9, 359p.

Diabate, S., Traore, A., Kone, B., 2010. Evaluation of the performance of tolerant crosses of oil palm selected in prenursery and replanted on wilt disease areas. Agric. Biol. J. North Am. 1(6): 1273-1277.

Durand-Gasselin, T., Cochard, B., Amblard, P., Nouy, B., 2009. Exploitation de l'heterosis dans l'amelioration genetique du palmier a huile (*Elaeis guineensis* Jacq.). Le selectionneur Français 60, 91-100.

Durand-Gasselin, T., de Franqueville, H., Diabate, S., Cochard, B., Adon, B., 2003. Assessing and utilizing sources of resistance to *Fusarium* wilt in oil palm (*Elaeis guineensis* Jacq.) genetic resource. Int. Symp “Oil palm genetic resources and utilization”. 8-10 June 2003, Malaysian Palm Oil Board, Kuala Lumpur.

Hartley, C.W.S., 1988. The Oil Palm (Tropical Agriculture Series), 3rd Edn. Longman Scientific & Technical, Harlow, London. 761p.

Hughes, G., Madden, L.V., Munkvold, G.P., 1996. Cluster sampling for disease incidence data. Letter to the Editor. Phytopathol. 86(2), 132-137.

Jain, J.K., Rastogi, R., 1998. Application of image processing in Biology and Agriculture. Nuclear India, pp. 12-13.

Jennifer, R., Aduwo, Ernest Mwebaze, 2004. Automated vision based diagnosis of cassava mosaic diseases. Virus Res. pp.129-142.

Kanchana, K.B., Manjula, W., Chinthika, P.G., Nilantha, R., Chamil, M., Neluka, F., 2015. Molecular characterisation and disease severity of *Leptospirosis* in Sri Lanka. Mem Inst Oswaldo Cruz, Rio de Janeiro, pp. 1-7.

Kover, P.X., Schaal, B.A., 2002. Genetic variation for disease resistance and tolerance among *Arabidopsis thaliana* accessions. PNAS 99 (17), 11270-11274.

Lindow, S.E., 1983. Estimating disease severity of single plants. Symposium: Estimating yield reduction of major food crops of the world. Phytopathol. 73(11), 1576-1581.

Madden, L.V., Hughes, G., van den Bosch, F., 2007. The Study of Plant Disease Epidemics. APS Press, St. Paul, MN.

Meunier, J., Gascon, J.–P., 1972. Le schema general d’amelioration du palmier a huile a l’IRHO. Oleagineux 27(1), 1-12.

Meunier, J., Renard, J.L., Quillec, G., 1979. Heredity of resistance to *Fusarium* wilt in the oil palm *Elaeis guineensis* Jacq. Oleagineux. 34, 555-61.

Nehal, S.E., Abdel-Kader, M.M., Lashin, S.M., Megahed, A.A., 2013. Fungicides alternatives as plant resistance inducers against foliar diseases incidence of some vegetables grown under plastic houses conditions. Int. J. Eng. Innov. Technol. 3(6), 71-81.

Ngando-Ebongue, G.F., Etta, C.E., Ntsomboh-Ntsefong, G., Oben, T.T., 2013. Breeding oil palm (*Elaeis guineensis* Jacq.) for *Fusarium* wilt tolerance: an overview of research programmes and seed production potentialities in Cameroon. Int. J. Agric. Sci. 3(5), 513-520.

Nita, M., Ellis, M.A., Madden, L.V., 2003. Reliability and accuracy of visual estimation of *Phomopsis* leaf blight of strawberry. Phytopathol. 93, 995-1005.

Noumouha, E.N., Ghislain, Allou, D., Adon, B., Konan, J.N., Diabate, S., Konan, K.E., Simon-Pierre, A., Nguetta, 2014. Assessment of Nigerian wild oil palm (*Elaeis guineensis* Jacq.) populations in crosses with Deli testers. J. Plant Breed. Genet. 2(2), 77-86.

Ntsomboh, N, G., Epoh Nguea, T., Madi-Galdima, Nsimi Mva, A., Ngando Ebongue, G.F., Kounga-Tagne, S., Mpondo-Mpondo, E., and Dibong, D., 2015. Isolation and *in vitro* characterization of *Fusarium oxysporum* f. sp. *elaeidis*, causal agent of oil palm (*Elaeis guineensis* Jacq.) vascular wilt. Res. Plant Sci. 3 (1), 18-26.

Ntsomboh, N.G., Ngando-Ebongue, G.F., Koona, P., Bell, J.M., Youmbi, E., Ngalle-Bille, H., Bilong, E.G., Madi, G., Anaba, B., 2012. Control approaches against vascular wilt disease of *Elaeis guineensis* Jacq. caused by *Fusarium oxysporum* f. sp. *elaeidis*. J. Biol. Life Sci. 3(1), 160-173.

Nutter, F.W. Jr., 1990. Remote sensing and image analysis for crop loss assessment. In: Crop Loss Assessment in Rice. International Rice Research Institute, Manila, The Philippines. pp.93-105.

Nutter, F.W., Jr., Teng, P.S., Shokes, F.M., 1991. Disease assessment terms and concepts. Plant Dis. 75, 1187-1188.

Prendergast, A.G., 1963. A method of testing oil palm progenies at the nursery stage for resistance to vascular wilt disease caused by *Fusarium oxysporum*, Schl. J. W. Afr. Inst. Oil Palm Res. 4, 156-175.

Renard, J.L., de Franqueville, H., 1989. Oil palm vascular wilt. Oléagineux 44(7), 341-349.

Renard, J.L., Gascon, J.P.,Bachy, A., 1972. Research on vascular wilt disease of the oil palm. Oleagineux. 27 (12), 581-591.

Renard, J.L., Meunier, J., 1983. Research for durable resistance to vascular wilt disease (*Fusarium oxysporum* f. sp. *elaeidis*) of oil palm (*Elaeis guineensis*). In: Durable Resistance in Crops (Eds.: Lamberto, F., Waller, J.M., Van der Graaf, N.A.). Plenum Publishing Corporation. pp.297-290.

Renard, J.L., Noiret, J.M., Meunier, J., 1980. Sources et gammes de resistance a la fusariose chez le palmier a huile *Elaeis guineensis* et *Elaeis melanococca* (Bilingue fr. – angl.). Oleagineux 35 (8-9) : 387-393.

Renard, J.L., Quillec, G., 1983. Fusariose et replantation. Elements a prendre en consideration pour les replantations de palmier a huile en zone fusariee en Afrique de l'ouest. Conseil de l'IRHO N^{o} 235, Oleagineux. 38(7), 421-427.

Renard, J.L., Ravise, A., 1986. La fusariose du palmier a huile. Phytoma. 374, 44-46.

Rival, A., Levang, P., 2014. Palms of controversies: Oil palm and development challenges. Bogor, Indonesia: CIFOR. Translated from Rival, A. and Levang, P., 2013. La palme des controverses: Palmier a huile et enjeux de developpement. Versailles, France: Editions Quæ.

Sanjay, B.P., Shrikant, K.B., 2011. Leaf disease severity measurement using image processing. Int. J. Eng. Technol. 3(5), 297-301.

Shen, W., Wu, Y., 2008. Grading method of leaf spot disease based on image processing. IEEE, pp.491-494.

Sungkur, R., Baichoo, S. et al., 2009. An automated system to recognize Fungi-caused diseases sugarcane leaves. Res. J. Univ. Mauritius 1-20.

Tengoua, F.F., 1993. Rapport du stage de formation a l'I.R.H.O. de Dabou (Côte-d'Ivoire) du 4 au 17 Juin 1993, 18p.

Tengoua, F.F., Bakoume, C., 2008. Pathogenicity of Cameroon strains of *Fusarium oxysporum* f. sp. *elaeidis* - the causal agent of oil palm vascular wilt. The Planter. 84 (985), 233-237.

Tucker, C.C., Chakraborty, S., 1997. Quantitative assessment of lesion characteristics and disease severity using digital image processing. J. Phytopath. 145, 273-278.

Wardlaw, C.W., 1946. A wilt disease of the oil palm. Nat. G.B. 158, 56.

Wijekoon, C.P., Goodwin, G.H., 2008. Quantifying fungal infection of plant leaves by digital image analysis using Scion Image Software. Elsevier, pp. 94-101.

William, W.H., Crosslacy, D.A., 1998. Video digitizer for the rapid measurement of leaf area lost due to herbivorous insect. J. Entomol. Society America, pp.591- 598.