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Research Article

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The Rain Forest Environment and Oil Palm Fatal Yellowing

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Abstract

Oil palm produces the most consumed vegetable oil in the world. In addition to being economically viable and having multiple uses, the crop has a strong ecological appeal, given its high level of carbon sequestration, its low environmental impact, and a low mechanization level in the harvest process, generating income and financial sustainability for the inhabitants of that biome. However, despite the large areas suitable for cultivation in harmony with the forest in Brazil, oil palm production has been limited by a disease known as fatal yellowing (FY). In 30 years of research to determine the causal agent of this disease, many epidemiology studies with insects and plant pathogens have been performed, but there is no consensus on its cause. Abiotic factors have also started to be considered a possible cause of these symptoms. Therefore, to clarify the relationship of this disease to environmental variables, we studied the nutritional status of the plant, the soil class and fertility, the climatic variables and attempted to verify the set of proteins with higher levels in diseased palms showing FY symptoms and healthy palms. FY occurred under constant rain and in clay soils; consequently, the roots of these palm trees suffered anoxia, which caused nutritional problems and the increase in levels of stress-related proteins. Under these conditions, the usually observed symptom was yellowing, which can lead to the death of the plant, giving the disease its name. In other words, the symptomatic picture is not associated with only a biotic cause. Thus, a pedological survey of the area, planting in soils not subject to waterlogging or with good drainage conditions, and the correct maintenance of soil fertility and plant nutrition may certainly contribute to the management and reduction of fatal yellowing without the use of agrochemicals.

Keywords: Elaeis Guineensis Jacq, Oil Palm, Precipitation, Soil Class, Proteomics

1. Introduction

The demand for vegetable oils is expected to reach 240 million tons by 2050 [1]. Therefore, to serve this market, the expansion of oilseed crops is necessary. Elaeis guineensis Jacq. Is considered the plant with the greatest economic viability that can achieve this goal because of factors such as high productivity, environmental and social sustainability, and low mechanization in the harvesting process, low production costs and the versatility of its products [2]. Oil palm plantations have the highest oil production per unit area in the world. One hectare planted with this palm can supply 3.74 tons of oil, while other oilseeds, such as soybean (0.38 ton/ha) and sunflower (0.48 ton/ha), have lower productivity than this specie [3, 4]. Given this potential, its production increased by 16.5% from 2012 to 2014, and it represented 39% of all vegetable oils consumed worldwide. The main countries responsible for supplying this demand for palm oil are Malaysia and Indonesia, representing 85% of the world market. Other countries such as Nigeria, Thailand, Colombia, Ecuador, and New Guinea together hold 6.6% of production [5]. Currently, Brazil has low representation in this sector; however, the country has 31.8 million hectares of suitable soil, sustainable agriculture and an ideal climate for planting oil palm, making it the country with the greatest potential for its cultivation [6]. In the Legal Amazon, approximately 29 million hectares may be used to grow oil palm [7]. In contrast, only 78 thousand hectares are being explored for this crop [8]. Although this region has good conditions for the cultivation of this palm, fatal yellowing (FY) limits its production and discourages producers from investing in planting this palm.

FY is considered the most important oil palm disease in Latin America, causing losses since 1967. In Pará, the largest Brazilian state producing palm oil, approximately 5,000 hectares have already been eradicated due to this disease, leading to hundreds of unemployed in the sector, as this crop uses intense labor in cultural practices and harvesting [9]. Its symptoms can be identified initially with the yellowing of the basal leaflets of the intermediate leaves, necrosis in its extremities, and their evolution to the total drought and death of the plant [10]. Although it is considered the largest phytosanitary problem of this palm, there is still no consensus on the causal agent of FY, making it difficult to implement measures for effective control [11].

Several attempts to identify the biotic causal agent of FY have been made in recent decades aiming to expand oil palm production in the country; however, they have failed in discovery the cause of FY. Among this studies, epidemiological studies were conducted addressing the spatial and temporal patterns of FY, not getting enough results to determine the etiology of the disease [12-14]. According to these studies, the spatial distribution of this disease is variable. FY starts with linear growth in incidence over time, changing to exponential over the years. Thus, both in the temporal and spatial analysis performed by Bergamin Filho et al. and Laranjeira et al., the progress of FY did not have a pattern consistent with those of other biotic diseases, and it is not possible to pinpoint a probable cause of the disease. In addition, recently, modern molecular fungal diversity techniques were used to attempt to identify and clarify the cause of the disease, and a specific etiologic agent for the origin of the disease was not found [15].

Accordingly, some studies have turned to the abiotic origin of FY, focusing on soil fertility and physical properties. According to Silva, the occurrence of FY coincided with soil patches with characteristics considered unsuitable for agriculture since they are subject to periodic flooding due to poor drainage [16]. Hartley found that approximately 60% of the oil palm field soil in Pará State, Brazil, should be avoided due to the edaphic characteristics inadequate for the cultivation of the palm [17]. The interaction of clay soils with poor rainfall distribution and heavy rainfall concentrated in the first months of the year increases the chances of flooding and high soil moisture.

In addition to soil classification and climatic conditions, the nutritional status of plants can also be associated with disease progress [18-20]. Crops planted in soils with good fertility and plants with balanced nutrition have lower losses in productivity and a lower incidence of diseases than crops planted in soils with nutritional limitations [21, 22]. This is because mineral elements are involved in the defense mechanisms of the plant, being components of cells and the cell wall, enzymes, inhibitors and regulators of secondary metabolites, and their deficiency damages the defense system of the plant [23]. Therefore, nutritional balance can be just as important to disease control as adequate soil fertility conditions and environmental variables.

As shown by Silva et al., the incidence of coffee brown eye spot is correlated with Ca and Mg imbalance and with the leaf nutrients B, K and P, as there was a higher occurrence of the disease in plants with lower levels of these nutrients in the leaves. The differences in the concentration of Ca and K in the healthy and diseased areas of coffee leaves were also demonstrated by Belan et al. [24]. Another example of a mineral element linked to disease control is silicon (Si); although Si is necessary for only some plant species, it can help in the management of diseases, forming physical barriers, preventing the penetration and colonization of pathogens in the host and activating defense biochemical mechanisms [25-27].

Consequently, in the face of factors such as waterlogging and nutritional deficiencies, plants cannot maintain their metabolic activities, as the root system cannot properly metabolize energy and undergoes fermentation [28, 29]. This anaerobic metabolism causes increased gene transcription of enzymes related to ethanol fermentation and increased production of carbohydrates to serve as substrates in this process [30]. Thus, identifying molecular changes in these plants can help in identifying the origin of the disease.

Nascimento et al., conducted the first study to describe oil palm root protein changes associated with FY [31]. The authors observed proteins related to anaerobic metabolism in all sampled plants, symptomatic or not, suggesting recent or constant hypoxia in their respective environments. In comparisons of the severity of FY symptoms in different stages, increased levels of alcohol dehydrogenase and energy-related proteins since the onset of symptoms contrasted with an increase in proteins related to biotic stress in the late stages of the disease. This finding suggests that changes in abiotic factors may precede the occurrence of FY and may also open the way for opportunistic pathogens.

Thus, considering the importance of the environment in the progression of diseases, the objectives of this study were to evaluate the relationship between the incidence of FY in oil palm and the nutritional status of the plant, the class and soil fertility, and the climatic variables, as well as to verify the set of proteins related to response to biotic and abiotic stimulus with higher levels in sick and healthy palm trees exposed to these environmental conditions.

2. Materials and Methods

2.1. Characterization of the Experimental Area

The progress of FY in oil palm (E. guineensis) was monitored visually in plantations located in the municipality of Mojú, Pará State, Brazil, where the climatic type is Am, tropical monsoon, according to the Köppen classification, with an annual average temperature of 25 to 27 °C, monthly insolation from 148.0 to 275.8 h, relative humidity of approximately 85%, total annual precipitation from 2000 to 3000 mm, and a rainy period from January to June [32]. The relief is flat, with small gaps varying from 0 to 3%, and the predominant soil is the yellow Latosol [33]. The disease progress data came from incidence surveys performed on all plants on Amanda´s farm, latitude 2° 06'43" South and longitude 48° 52'38" West, and Bethânia´s farm, latitude 2° 06'43" South and longitude 48° 48'18" West, with 9,984.66 and 119.14 ha, respectively, totaling 527 plots of variable size.

2.2. Data collection, incidence frequency intervals and area under the progressive disease curve (AUPDC)

The assessments were made monthly between 2012 and 2015. In each plot, the number of new sick plants was observed, and the position of the tree and the date of the first symptoms were recorded. The incidence of FY was calculated according to the equation [34]:

I (%) = NDP/NPT*100

Therefore:

I (%) = incidence of FY,

NDP = number of diseased palms,

NPT = total number of palm trees sampled.

Annual histograms were then constructed with cumulative frequencies of 1% intervals and new occurrences with 10% intervals. In addition, the data obtained were integrated into the area under the disease progress curve metric according to the equation proposed by Shaner and Finney [35].

$$AUPDC = \sum[((Y_i + Y_{i+1})/2) * (t_{i+1} - t_i)]$$

Therefore:

AUPDC = area under the progress curve of the incidence of FY,

Y_i = proportion of the disease in the i-th observation,

T_i = time in days in the i-th observation,

n = total number of observation.

2.3. Relationship of Climatic Variables with Oil Palm FY

The climatic variables were obtained from the INMET (Instituto Nacional de Meteorologia) climatological stations located in the Belém and Cametá municipalities, Pará State, Brazil. The data analyzed were averages of maximum and compensated temperatures (°C), relative air humidity and cloudiness (tenths) (%), precipitation (mm), average and maximum wind speeds (m/s), insolation (hs), tar evaporation (mm), potential and real evapotranspiration (mm).

The analyses were carried out jointly, with data from the two properties, as the study took into account meteorological variables of regional scope.

2.4. Relationships of classification, soil fertility and plant nutrition with the progress of FY in oil palm

The collection of soil and leaves was carried out in 2013 and 2014. Leaf tissue was sampled in each plot in the two properties randomly in 10 plants in the middle third of each leaf, with 4 leaflets per plant being collected. The tissues of the aerial parts were weighed to obtain the weights of these fresh organs. Subsequently, they were stored in an oven at 65 °C, and their dry weights were obtained. The dry samples were ground and weighed to 1 gram and then used to determine the levels of leaf nutrients according to the methodology described by Malavolta et al. [36]. The reference values for interpreting the results of the leaf analysis were based on Guzmán for the oil palm culture (Table 1) [37].

Macronutrient	Range	Micronutrient	Range
N	19.62 - 28.81 g/kg	Cu	4.20 - 7.50 mg/kg
P	1.25 - 1.88 g/kg	Zn	12.40 - 20.60 mg/kg
K	7.98 - 12.10 g/kg	B	9.60 - 17.30 mg/kg
Ca	6.26 - 7.91 g/kg		
Mg	1.76 - 2.90 g/kg		
S	1.19 - 1.86 g/kg		

Table 1: Reference Values for Leaf Content of Macronutrients and Micronutrients for Oil Palm According to Guzmán (2014).

To obtain fertility in the sampled areas, the soil from each plot was collected from a composite sample of at least 12 points at a depth of 0 to 20 cm. These samples were sent to the laboratory and placed in 50 ml polypropylene digestion tubes with 5 ml of concentrated nitric acid at 115 °C for 45 min, which was maintained using a hot block (DigiPREP System®). The levels of K, Ca, Mg, B, Cu, Mn, Zn and Fe were quantified by optical emission spectroscopy with inductively coupled plasma (ICP-OES) in an iCAP 6500 instrument (Thermo Fisher Scientific®, Waltham, MA).

To assess the influence of soil classes on the accumulated incidence of FY in the years 2013 and 2014, soil classes obtained in a pedological survey carried out previously were selected, with the highest occurrence at the Mojú-Pa pole and with the greatest contrast between the quantities of clay and sand in horizons A and B. The selected classes were PACal md/arg (Gray Acrisols, medium/clayey texture), PACd md/arg (DYL-Gray Acrisols, medium/clayey texture), PACal + PACd md/arg (alkyd Gray Acrisols + Dystrophic Gray Acrisols, medium/clay texture), ESPd (dystrophic Podzols), RQGal (alkyd Arenosols) and RQd (dystrophic Arenosols).

2.5. Statistical Analysis

The assumptions for the analysis of variance for AUPDC and accumulated and new cases of incidence were verified with the Shapiro-Wilk and Bartlett tests to verify their normality and homogeneity, respectively. The incidence data and AUPDC were submitted to analysis of variance, and the significance was verified by the F test ($p < 0.05$). The results for the average disease incidence index were plotted on graphs of the disease progress curve, along with the averages of the climatic variables. The data were analyzed, considering the monthly progress curves separately, according to the years with the highest intensity of the disease (January/December 2013 and January/December 2014). Annual histograms were constructed, with frequencies ranging from 1 to 53% of incidence in the field to accumulated incidence and from 0 to 70% for new occurrences of the disease for all years. A histogram for the frequencies of plants with deficiencies and excesses of the nutrients N, P, K, Ca, Mg, S, Zn, Cu and B was constructed with the leaf nutrition data from 2014. To determine the correlations between the monthly incidence of the disease and the climatic variables and the relationships between AUDPC, soil fertility and plant nutrition, Pearson's correlation analysis was used ($p < 0.05$).

The incidence data were related to the monthly averages of the climatic variables (nonlagged) and to the averages of the variables of the thirty days prior to the field incidence assessments (lagged), regardless of the day of the evaluation in the month. In each soil class, the percentages of plots in the two properties, Amanda and Bethânia, were evaluated with accumulated incidence intervals of 0-1, 1-10, 10-20 and 20-30% of the new cases in 2013 and 2014. All analyses were performed using R software (R Core Team 2013).

2.6. Proteomic Analysis

Sampling was carried out in field conditions in February 2017, during a period of higher rainfall, in a sandy yellow dystrophic latosol in a farm also located in municipality of Mojú, Pará state, in northern Brazil. Roots of asymptomatic (Asy) and symptomatic (FY, fatal yellowing) plants in the intermediate stage of FY were collected according to the classification proposed by Souza et al. [38]. After washing with water, the roots were kept in liquid nitrogen and transported to the laboratory of Instituto Tecnológico Vale – Desenvolvimento Sustentável.

Roots from fifteen plants were pooled to obtain three biological replicates for each condition, and each replicate consisted of five plant roots (obtaining 3 g of fresh matter for each replicate). Proteins were isolated following the SDS (sodium dodecyl sulfate)/phenol protocol proposed by Wang, and their concentrations were measured on a Qubit 2.0 fluorometer (Invitrogen, Thermo Fisher Scientific) using a Qubit protein assay kit according to the manufacturer's protocol [39]. For protein digestion, 50 µg of protein was used from each sample for an in-solution digestion and desalting protocol according to Nascimento et al.

An aliquot containing 4.5 µg of each digested protein sample was loaded for separation with a Nano Acquity UPLC® System (Waters Corp.) equipped with 2D online dilution technology. The peptide fraction was separated along the first chromatographic dimension under basic (pH = 10) conditions in a BEH C18 300 Å, 5 µm 300 µm x 50 mm reversed-phase column (XBridgeTM, Waters Corp.). This was performed at a flow rate of 2 µL/min. Eluent A was aqueous 20 mM FA (pH = 10), and eluent B was neat ACN. All samples were analyzed using a five-step fractionation method. The fractions were eluted from the first dimension using a composition of 10.8, 14.0, 16.7, 20.4 or 65% eluent B.

The fractionation process was programmed to start immediately after completion of sample loading (20 min at 10 µL/min with 3% B). Each first-dimension elution step was performed with a 20 min run time using a flow rate of 2 µL/min. The eluent peptide was mixed online with 10 µL/min of 0.1% TFA solution (1:10 dilution) before being trapped in the trapping column (100 µm x 100 mm), which was packed with 1.7 µm 100 Å silica-based C18 (Symmetry, Waters Corp, Milford, MA).

The mobile phase for the second chromatographic dimension (low pH RS) was 0.1% FA in water (immobile phase A) and 0.1% FA in ACN (mobile phase B). The second-dimension column was 100 µm x 10 mm C18 packed with changed surface hybrid (CSH) 1.8 mm particles (Acquity UPLC M-Class CSH C18, Waters Corp., Milford, MA). The flow rate for the second-dimension separation was 400 nL/min, while the column was maintained at 55 °C. A 40 min gradient from 3 to 40% B was used to separate peptides in the second separation dimension. The column was then washed using 90% B for 1 min and equilibrated with 3% B for 7 min before returning to the next fractionation step.

Mass spectra were obtained with a Synapt G2-S spectrometer equipped with a standard electrospray ionization (ESI) source (Waters). For all measurements, the mass spectrometer was operated in positive ion resolution mode. Mass spectra were acquired in continuum mode over an m/z range of 50-1200 using a capillary voltage of 3 kV, source temperature of 100 °C, source offset voltage of 30 V, cone gas flow of 50 L/h and cone voltage of 30 V. The spectral acquisition time at each energy setting was 0.5 s. A solution of 0.2 µM Glu1-fibrinopeptide (785.8427 Da) was used as a lock-mass solution and delivered at a flow rate of 0.5 µL/min using an auxiliary pump of the liquid chromatography system. The lock mass was sampled every 30 s using 0.1-s scans over the same mass range.

The data were processed using the Progenesis QI software (Waters) for identification and quantification, using the Viridiplantae database from uniprot (uniprotkb/swiss-prot, uniprot.org). Protein identification was accepted only if the probability of identifying peptides was greater than 90 %, and proteins with 95 %. The significance levels of the differential abundances of proteins were determined by applying the ANOVA test ($p < 0.05$). In order to compare the proteome of plants with and without symptoms of fatal yellowing, a principal component analysis (PCA) of the proteins with differential abundance and with p < 0.05 was produced in the R software v3.6.3 (R Core Team 2018; https://www.R-project.org), using packages FactoMineR, Factoshiny and factoextra. The Gene Ontology (GO) analyses of proteins were performed using the OmicsBox v1.2.4 (bioBam) and Uniprot (uniprotkb/swiss-prot, uniprot.org) database. Heatmaps were developed using R software v.3.6.3 (package pheatmap v.1.0.12, ggplot2 v.3.3.5, colorspace 2.0-2 and grid 4.0.4).

3. Results

3.1. Analysis of Frequency Ranges

Over the years evaluated, there was significant disease progression over time ($p<0.05$). In addition, there was variation in the mean disease intensity among the evaluation periods. In 2012, approximately 5% of the plots were evaluated with a 0% incidence, while in 2015, there were symptoms of FY in all plots (Table 2). The numbers of plots with an incidence of 0.1 to 1% changed from 81.8% in 2012 to 7.6% in 2014, with progress being made for the frequencies with the highest percentage of FY, as in the intervals from 1 to 15% (Table 2).

In an analysis of the histograms, migration of classes 0.1-1 and 1.1-2% of incidence occurred for intervals of 3 to 54%, with frequency distributions with a tendency to the right of the centers throughout the evaluated period (Figure 1).

In an analysis of new cases of FY, the percentage of plots in the class of 1 to 10% decreased from 94.1 to 84.8% in 4 years, while in the range of 11-20%, it increased from 0.4 to 10.1%. In 2014 and 2015, parcels in the ranges of classes above 40% were observed (Figure 2).

% Incidence	Percentage of plots for range of incidences				% Incidence	Percentage of plots for range of incidences			
	2012	2013	2014	2015		2012	2013	2014	2015
0	5.3%	1.7%	0.6%	0.0%	31.1 – 32	0.0%	0.4%	0.2%	0.0%
0.1 – 1	81%	32.3%	12.5%	7.6%	32.1 – 33	0.0%	0.2%	0.4%	0.0%
1.1 – 2	7.8%	28.5%	23.0%	18.8%	33.1 – 34	0.0%	0.2%	0.4%	0.2%
2.1 – 3	0.9%	11.2%	16.9%	16.9%	34.1 – 35	0.0%	0.0%	0.0%	0.0%
3.1 – 4	0.4%	13.7%	11.0%	13.7%	35.1 – 36	0.0%	0.2%	0.0%	0.0%
4.1 – 5	1.3%	4.7%	7.4%	6.8%	36.1 – 37	0.0%	0.0%	0.2%	0.4%
5.1 – 6	0.9%	1.5%	7.6%	5.7%	37.1 – 38	0.0%	0.4%	0.4%	0.0%
6.1 – 7	0.4%	0.8%	5.5%	4.0%	38.1 – 39	0.0%	0.0%	0.4%	0.2%
7.1 – 8	0.2%	0.6%	3.0%	4.2%	39.1 – 40	0.0%	0.0%	0.0%	0.4%
8.1– 9	0.6%	0.2%	2.7%	4.9%	40.1 – 41	0.0%	0.0%	0.2%	0.2%
9.1 – 10	0.0%	0.2%	1.3%	2.3%	41.1 – 42	0.0%	0.0%	0.2%	0.0%
10.1 – 11	0.0%	0.0%	1.5%	1.5%	42.1 – 43	0.0%	0.0%	0.0%	0.4%
11.1 – 12	0.2%	0.2%	0.8%	2.1%	43.1 – 44	0.0%	0.0%	0.0%	0.2%
12.1 – 13	0.0%	0.0%	1.1%	2.3%	44.1 – 45	0.0%	0.0%	0.0%	0.0%
13.1 – 14	0.0%	0.2%	0.2%	1.1%	45.1 – 46	0.0%	0.0%	0.0%	0.4%
14.1 – 15	0.0%	0.0%	0.2%	0.6%	46.1 – 47	0.0%	0.0%	0.2%	0.0%
15.1 – 16	0.0%	0.0%	0.0%	1.3%	47.1 – 48	0.0%	0.0%	0.0%	0.2%
16.1 – 17	0.0%	0.2%	0.0%	0.2%	48.1 – 49	0.0%	0.0%	0.6%	0.0%
17.1 – 18	0.0%	0.0%	0.0%	0.4%	49.1 – 50	0.0%	0.0%	0.8%	0.2%
18.1 – 19	0.0%	0.0%	0.0%	0.4%	50.1 – 51	0.0%	0.0%	0.2%	0.0%
19.1 – 20	0.2%	0.6%	0.2%	0.2%	51.1 – 52	0.0%	0.0%	0.0%	0.2%
20.1 – 21	0.0%	0.2%	0.0%	0.0%	52.1 – 53	0.0%	0.0%	0.0%	0.6%
21.1 – 22	0.0%	0.2%	0.0%	0.2%	53.1 – 54	0.0%	0.0%	0.0%	0.6%
22.1 – 23	0.0%	0.4%	0.0%	0.2%	54.1 – 55	0.0%	0.0%	0.0%	0.0%
23.1 – 24	0.0%	0.2%	0.2%	0.0%	55.1 – 56	0.0%	0.0%	0.0%	0.0%
24.1 – 25	0.0%	0.4%	0.2%	0.0%	56.1 – 57	0.0%	0.0%	0.0%	0.0%
25.1 – 26	0.0%	0.0%	0.0%	0.0%	57.1 – 58	0.0%	0.0%	0.0%	0.0%
26.1 – 27	0.0%	0.4%	0.2%	0.2%	58.1 – 59	0.0%	0.0%	0.0%	0.0%

27.1 – 28	0.0%	0.0%	0.0%	0.0%	59.1 – 60	0.0%	0.0%	0.0%	0.0%
28.1 – 29	0.0%	0.0%	0.0%	0.0%	60.1 – 61	0.0%	0.0%	0.0%	0.2%
29.1 – 30	0.0%	0.0%	0.0%	0.0%	61.1 – 62	0.0%	0.0%	0.0%	0.0%
30.1 – 31	0.0%	0.6%	0.0%	0.2%	62.1 – 63	0.0%	0.0%	0.0%	0.2%

Table 2: Frequency of New Occurrences of Fatal yellowing on Palms between 2012 and 2015 in Mojú Municipality, Pará State, Brazil, Separated into Classes of 1% Incidence.

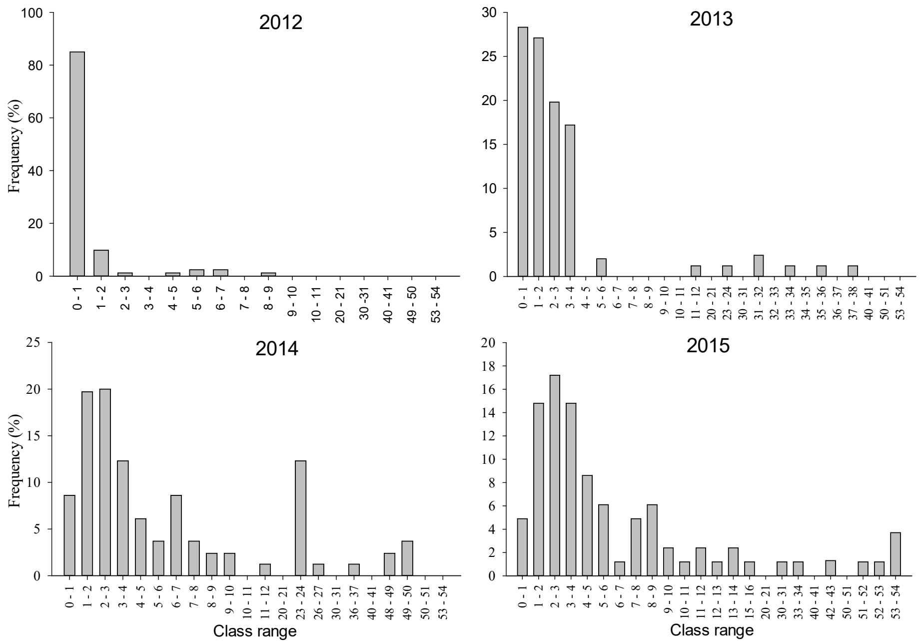

Figure 1: Distribution of the Accumulated Frequency Classes (with 1% intervals) of Fatal Yellowing between 2012 and 2015.

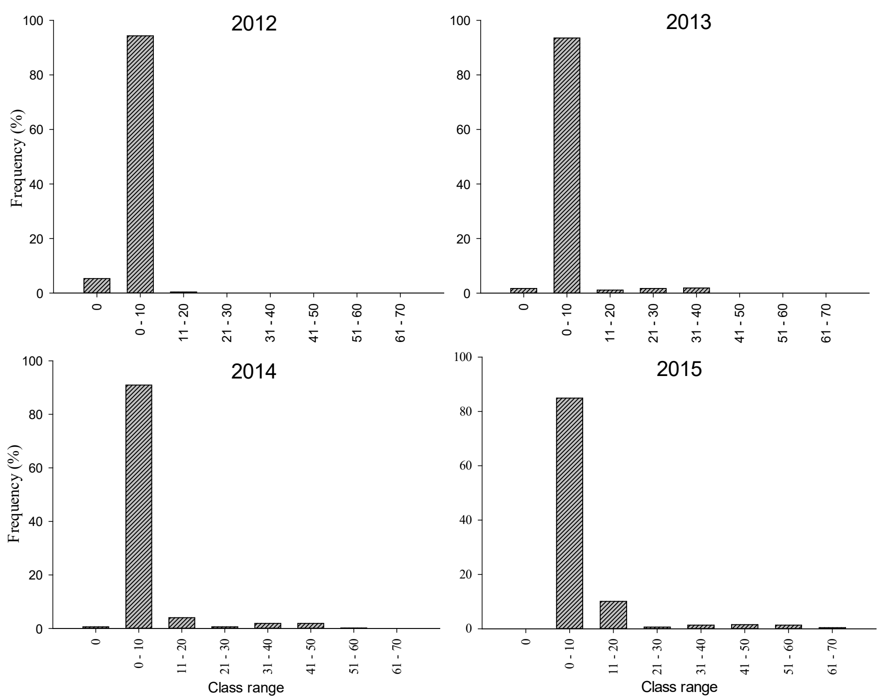

Figure 2: Distribution of Classes of New Occurrences (with 10% intervals) of Fatal Yellow between 2012 and 2015.

3.2. Relationship of Climatic Variables in Rain Forest With Oil Palm FY

There was variation in the average disease intensity among the assessment periods. However, the years with the highest disease incidence were 2013 and 2014. In the average progress curve for 2013 of new plants with FY, there was an increase in the incidence from February to May, coinciding with increases in the rainfall and relative humidity to 500 mm and 86%, respectively. The maximum value of the average incidence was 7.95% in May. In the following months, there was a reduction in the disease incidence values, with decreases in the rainfall (180 mm) and relative humidity (80%) and increases in the average wind speed (2 m/s), real evapotranspiration (123 mm) and tar evaporation (64 mm) (Figure 3).

In 2014, the disease incidence of new cases also increased in February (3.2%), being lower than that observed in 2013. The increase in new cases was greater in the first half of the year. In this interval, there was also greater accumulated monthly rainfall, with values of 476, 505, 482, 346 and 231 mm for the months of February, March, April, May and June, respectively. Consequently, there was a higher relative humidity of the air in the same interval, with the highest percentage in February (89%). In the dry months, relative humidity and precipitation decreased, reaching 76% and 78 mm, respectively, in October. In June, with the reduction in rainfall, the average temperature in the second half of the year was 33 °C, resulting in increased evaporation of water from the soil. With the lower availability of water in the soil, there was a reduction in evapotranspiration and in the incidence of FY (Figure 3).

For the nonlagged monthly data for 2013, the variable "real evapotranspiration" had a significant positive correlation with the incidence frequency for new cases of FY (Table 3). The variable "maximum wind speed" had a significant negative correlation with this incidence frequency. For the other meteorological variables, there was no significant correlation with the disease for the months of 2013 (Table 3). According to outdated monthly data for 2013, the variables "precipitation" and "relative air humidity" had a positive correlation, while the variables "tar evaporation" and "average wind speed" had a negative correlation with the FY (Table 3).

For the nonlagged monthly data for 2014, only the variable "real evapotranspiration" had a significant positive correlation with the incidence of new cases of FY from oil palm. However, when the data lagged, all meteorological variables correlated significantly with the incidence of FY. The variables "relative air humidity", "cloudiness", "real evapotranspiration" and "precipitation" had a positive correlation with the FY of the oil palm, with Pearson's correlation values of 0.42, 0.37, 0.35 and 0.31, respectively (Table 3).

In contrast, the variables "maximum wind speed", "tar evaporation", "average wind speed", “maximum wind speed”, "potential evapotranspiration", "insolation", "maximum temperature" and "average temperature" had negative correlations with the disease (Table 3).

Climatic variable	Monthly Incidence 2013	Monthly Incidence 2013 lagged	Monthly Incidence 2014	Monthly Incidence 2014 lagged
Precipitation (mm)	0.08	0.26*	-0.06	0.31*
Humidity (%)	0.01	0.23*	0.08	0.42*
Cloudiness (dec)	-0.08	0.1	-0.08	0.37*
Real evapotranspiration (mm)	0.28*	0.06	0.29*	0.35*
Potential evapotranspiration (mm)	0.19	-0.18	0.07	-0.33*
Tar evaporation (mm)	-0.05	-0.27*	-0.1	-0.48*
Average wind speed (m/s)	-0.21	-0.28*	-0.17	-0.39*
Maximum wind speed (m/s)	-0.27*	-0.14	-0.15	-0.56*
Average temperature (°C)	-0.07	-0.13	0.1	-0.25*
Maximum temperature (°C)	0.08	-0.06	0.08	-0.27*
Insolation (h)	0.17	-0.03	0.18	-0.27*

* Significant at the 5% probability level

Table 3: Correlation between New Cases of Fatal Yellow Incidence in Oil Palm in 2013 and 2014 with the Average Meteorological Variables Collected at Belém and Cametá.

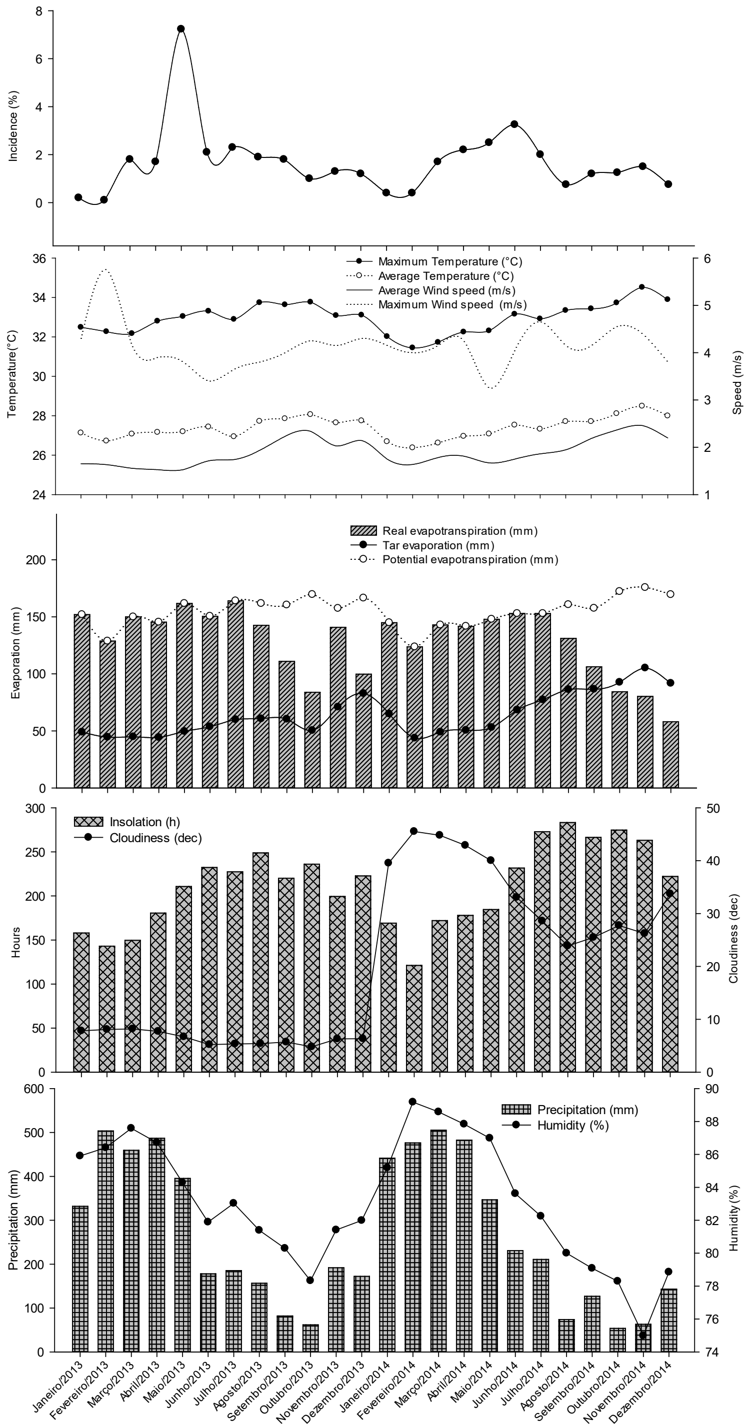

Figure 3: Progress curve of new cases of fatal yellow incidence in 2013 and 2014, total precipitation (mm), relative humidity (%), actual and potential evapotranspiration (mm), tar evaporation (mm) and maximum and average wind speeds (m/s), obtained from the weather stations in the municipalities of Belém-PA and Cametá-PA.

3.3. Relationship of Soil Classification and Fertility and Plant Nutrition with the Progress of FY in Oil Palm

There was a significant correlation between soil fertility and plant nutrition with FY incidence of oil palm. In 2013, the nutrient Ca had a significant negative correlation (-0.74) with soil fertility. Because micronutrients Mn and Fe had positive correlations (0.64 and 0.48), there was a higher incidence of FY in areas with higher levels of these nutrients in the soil. In 2014, the same pattern was observed, but K had a positive correlation and P, Mg and B had negative correlations with incidence (Table 4).

According to Guzmán (2014), the frequencies of plants deficient in the nutrients N, P, Ca, Mg, S, Zn and Cu were 50, 37, 49, 66, 50, 50 and 46%, respectively. Sixty-five percent and 46% of the plants had excess K and B, respectively (Figure 4). The correlation between Ca and AUCPD was significantly negative in 2013, while in 2014, in addition to Ca, the nutrients N, Si, S, B, Mn and Fe also had significant negative correlations with AUDPC, while K correlated positively (Table 5).

Soil nutrients	AUDPC	
	2013	2014
Water pH	0.05	0.13
P Mehlich	0.26	-0.67*
K	0.08	0.37*
Ca	-0.74*	-0.49*
Mg	-0.19	-0.56*
B	-0.24	-0.53*
Cu	0.16	0.07
Mn	0.64*	0.51*
Zn	0.03	0.12
Fe	0. 48*	0. 71*

* Significant at the 5% probability level

Table 4: Correlation of Soil Fertility with the Area under Disease Progress Curve (AUDPC) of the Fatal Yellow in 2013 and 2014.

Leaf nutrient	AUCPD	
	2013	2014
N	0.03	-0.47*
P	0.18	0.27
K	0.35	0.59*
Ca	-0.48*	-0.54*
Mg	0.06	-0.31
S	0.35	0.17
Si	-0.37	-0.56*
B	-0.25	-0.62*
Cu	0.02	0.22
Mn	0.34	-0.53*
Zn	0.14	0.09
Fe	0. 36	-0. 45*

Table 5: Correlation of Plant Mineral Nutrition with the Area under Disease Progress Curve (AUDPC) of the Fatal Yellow Incidence in 2013 and 2014.

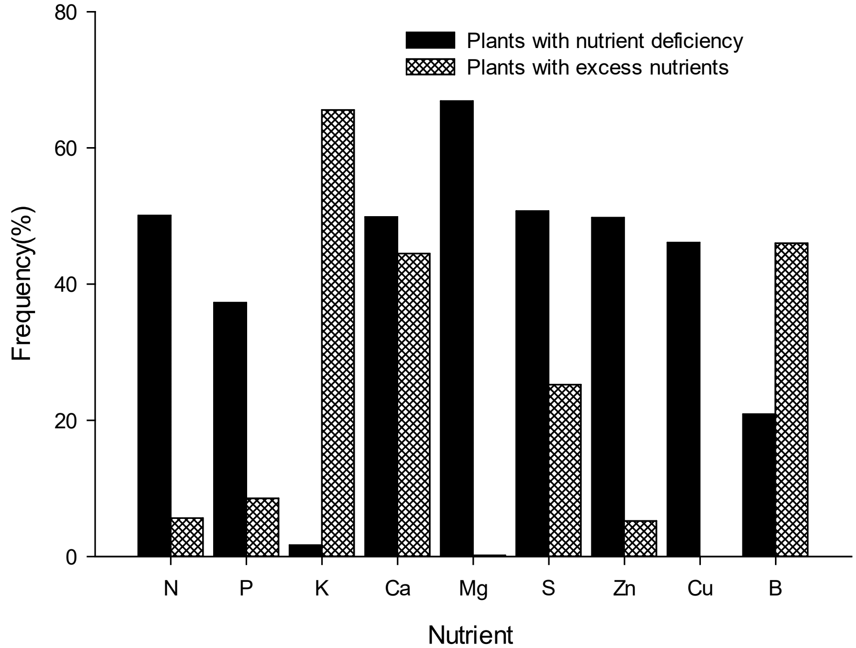

Figure 4: Percentages of plants with nutrient deficiency and excesses in 2014 in Mojú-PA.

The soil classes with the highest amount of clay, specifically the PAC or Grayish Argisol (Ultisol), had the highest occurrences of FY in all intervals of incidence of the disease in both 2013 and in 2014 (Figure 5). In 2013, a total of 10.9% of the plots classified as PACal md/arg had an incidence from 0-1%, while only 1.8% were from 20-30%. In 2014, the number of plots in the soil class from 0-1% decreased to 9.5%, while it increased to 3.6% in the 20-30% range of incidence. Sandy soils also had an increase in incidence. However, for clayey soil, the frequency of plots was lower.

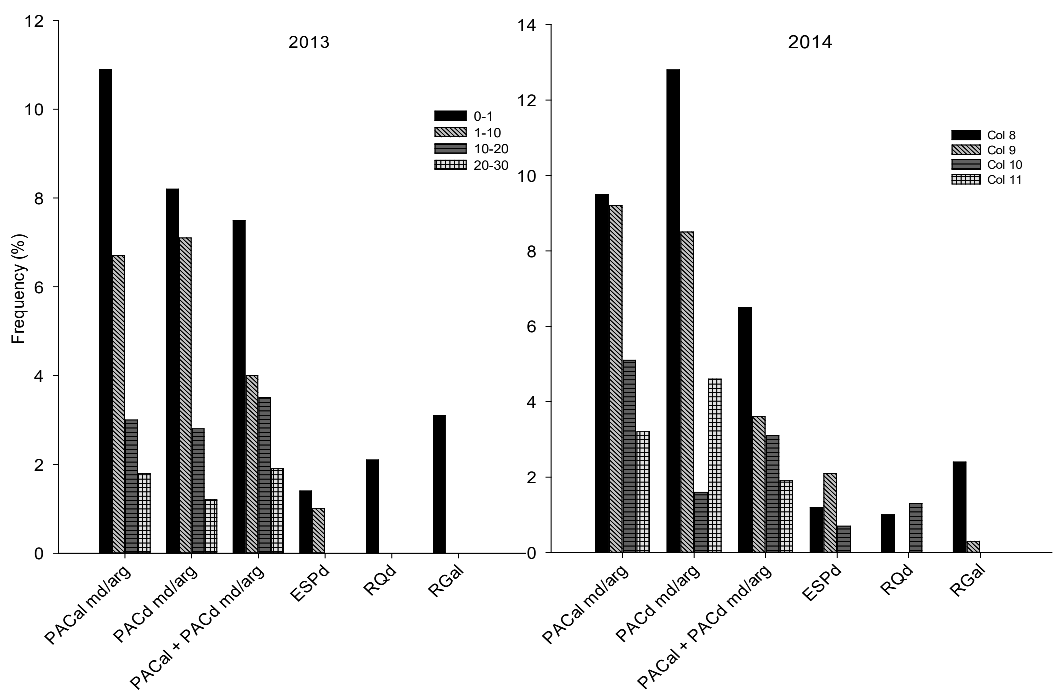

Figure 5: Plot Frequencies in Each Incidence Interval According to the Soil Class in 2013 and 2014.

3.4. Protein Profile

A total of 669 proteins were identified in FY and Asy samples, of which 81 presented p-value ≤ 0.05 and FC ≥ 1(Dataset Sx1), showing differential abundances. Of these proteins, 47 were upregulated in FY plants, while 34 were downregulated (Figure 6a), presenting higher levels in asymptomatic plants. The PCA of the 81 proteins differentially abundant with significant values showed the separation between root proteomes of plants with and without FY symptoms (Figure 6b).GO analysis in the Biological process category assigned the FY plants proteins to 43 GO terms, while asymptomatic plant proteins were assigned to 57 GO terms (Figure 7a). Distributed among a total of 62 GO terms, the proteins from the dataset were also included in the terms Primary metabolic process and Response to stress. New GO analyzes categorized the proteins attributed to these two biological processes into GO terms shown in the subgraphs of Figures 7b and 7c.

Primary metabolic process was composed of 25 proteins in plants with FY symptoms and 23 proteins in asymptomatic plants, including proteins involved primarily in the metabolism of carbohydrates, lipids, and metabolism of other proteins (Figure 7b). The GO analysis showed that proteins attributed to the Response to stress are involved in at least 11 processes in plants with FY, and in at least 12 processes in asymptomatic plants, highlighting processes related to the response to temperature, flooding, hypoxia, osmotic stress, oxidative stress, starvation and water deprivation (Figure 7c). Among the proteins attributed to the abiotic stress response in plants with FY are alcohol dehydrogenase 1 (P12886, Q2R8Z5 and P14219) and 2 (P28032), peroxidase 2 (A2YPX3), 1-Cys peroxiredoxin (P52571), calcineurin B-like protein 10 (Q7FRS8), caltractin (P41210), steroid 5-alpha-reductase DET2 (Q2QDF6), nodulin-related protein 1 (Q9ZQ80) and (Q94K66), translationally-controlled tumor (Q9ZSW9) (Figure 7c).

These stress-response proteins were also attributed to Defense response, where proteins involved in the biotic stress response were included. Thus, a GO analysis was applied to verify which defense processes these proteins are involved. Among the GO terms that the Defense response proteins have been assigned are responses to fungus and bacterium (Figure 7d). The highest amount of proteins upregulated in FY plants was attributed to the Defense response to fungus, which included the endochitinase CH25 (Q09023), arginase (O49046) serine/threonine-protein kinase BSK8 (Q9FHD7), phosphatase 2C 59 (Q8RXV3), glucan endo-1_3-beta-glucosidase GI (P34742) and 1-Cys peroxiredoxin (P52571) (Figure 7d). On the other hand, DNA damage-binding protein 1a (Q9M0V3), Transcription factor MYB93 (Q9S9Z2), Wall-Associated receptor kinase-like 20 (Q9LZM4) and peroxidase 22 (P24102) have been attributed to the Defense response to fungus in asymptomatic plants (Figure 7d). Nodulin-related protein 1 (Q9ZQ80) and phosphatase 2C 59 (Q8RXV3) were also upregulated and attributed to the Defense response to bacterium in FY plants (Figure 7d). In Asy plants, Defense response to bacterium was composed of the Transcription factor MYB93 (Q9S9Z2), peroxidase 22 (P24102) and peroxisomal (S)-2-hydroxy-acid oxidase proteins (Q01KC2) (Figure 7d). Proteins from plants with FY symptoms were also attributed to Innate immune response, Systemic acquired resistance and Defense response to symbiont, and the GO terms Defense response to nematode and Defense response to insect were identified only in asymptomatic plants (Figure 7d).

In general, proteins related to response to abiotic and biotic stimuli were downregulated in plants with FY symptoms, as can be seen in the heatmaps showing separately the proteins assigned to Primary metabolic process (Figure 7b), Response to stress (Figure 7c) and Defense response (Figure 7d). The groups of upregulated proteins with greater consistency in plants with FY compared to asymptomatic plants were attributed to the response to oxidative stress, hypoxia and flooding (Figure 7c). The hierarchical clusters separated the proteins into two well-defined groups in each heatmap (Figures 7b, 7c and 7d), where one group is formed by upregulated proteins in roots of FY plants and the other by proteins downregulated in these plants. Replicates of plants with and without FY symptoms were equally clustered in two groups.

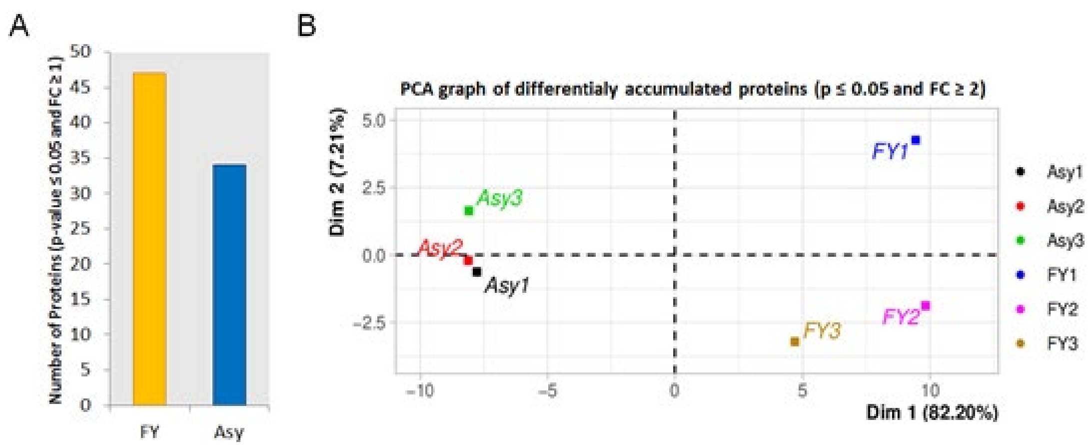

Figure 6: Differently abundance of proteins and PCA from the proteomes of oil palm roots of FY and Asy plants. A) Proteins most abundant in roots of plants with and FY without symptoms considering p-value ≤ 0.05 and Fold Change ≥ 1. B) PCA of proteins with differential abundance with p-value ≤ 0.05 and Fold Change ≥ comparing the proteome of plant roots without symptoms (Asy1, Asy2 and Asy3) and with symptoms of FY (FY1, FY2 and FY3). The proteins used for analysis are presented in Dataset Sx.

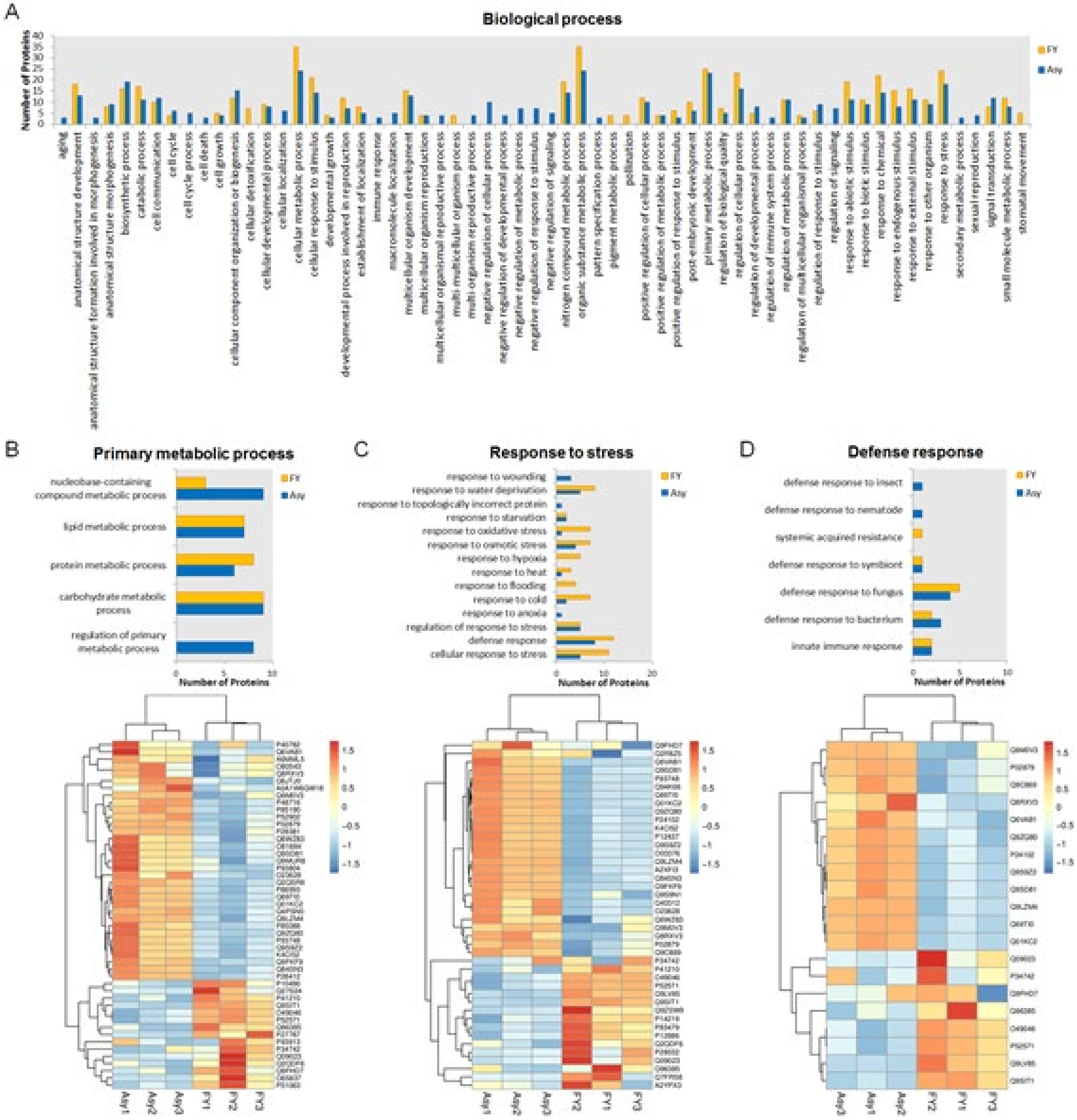

Figure 7: Gene Ontology (GO) annotation and Hierarchical clustering analysis of proteins with differential abundance identified in oil palm roots of FY and Asy plants. A) GO annotation showing the GO terms in the category of Biological Process of abundant proteins in plants with and without FY. B) Above a subgraph of the term Primary metabolic process and its set of proteins shown in a heatmap below. C) Above a subgraph of the term Response to stress and its set of proteins shown in a heatmap below. D) Above a subgraph of the term Defense response and its set of proteins shown in a heatmap below. The red and blue colors in the heatmaps represent the highest and lowest intensity values, respectively. The proteins used for analysis are presented in Dataset Sx.

4. Discussion

Relationships of FY in oil palm with environmental variables were verified in this work. The incidence of FY increased over the years evaluated, and its progress was evident in all plots of the two farms, in different percentages depending on the plot. In addition, incidence class intervals above 10% increased in 2014 and 2015 in the analysis of new cases of the disease. This result is similar to those from studies on the progression of the incidence of FY found in the literature. Bergamin Filho et at., analyzed cumulative FY incidence curves constructed with monthly and annual data, noting an increase in the incidences in the proportions of 0.40, 0.15 and 0.016 for plantations of 19, 14 and 9 years, respectively. As in the present study, there were plots with up to 50% accumulated incidence, and the economic viability of planting was impaired, so clarifying the cause of this disease is essential to establish management practices. Therefore, an analysis of environmental variables in Pará State, a region where the occurrence of this disease is common, and the plant's nutrition was performed to determine their correlation with FY.

The northern region of Brazil has the ideal climatic conditions for oil palm cultivation, as it requires some specific edaphoclimatic variables for satisfactory production. The ideal precipitation regime for this crop is an average annual rainfall of 1.800 mm. Another requirement is solar radiation. The necessary insolation to reach the productive potential of the oil palm is approximately 1.800/h/year, with a minimum of 5 h/day. In addition, greater productivity occurs in regions with small variations in temperature and annual averages between 25 and 27 °C [40]. However, these same factors promote several phytosanitary problems. According to the results obtained, higher precipitation intensity results in greater relative humidity of the air (83 to 89%), less evapotranspiration, and greater accumulation of water in the soil; these conditions and lower wind speeds provide a greater probability of FY occurrence. Thus, although oil palm requires 150 mm of precipitation per month, in this region, it can receive to 500 mm/month in the first half of the year. In months of the year with strong wind conditions (2 to 2.5 m/s), which is associated with high insolation values (220 to 250 h/month), i.e., reduced cloud cover and reduced rainfall and temperature (28 °C), there was less progress of the disease. Consequently, with greater insolation and presence of winds, greater evaporation and reduction in the amount of water in the soil occurs. The importance of water balance has also been studied by Venturieri et al., who showed that it has a negative correlation ($p = 0.0002$) with fatal yellow. In regions with mild water deficiency, no cases of this disease were observed.

In this work, the correlation of climatic variables with the incidence of FY was verified. There was a correlation between only actual evapotranspiration and wind speed in 2013 and 2014. However, when the data were lagged by thirty days immediately prior to the assessment of the disease, all climatic variables correlated with FY in 2014, indicating the importance of this approach. The lag in climatic data shows that a time interval is necessary for the plant to develop symptoms. Therefore, the manifestations of physiological imbalance in the field occurred due to intense rain and high relative air humidity thirty days immediately before the evaluations. To verify the morphological changes of water stress in the physiology of oil palm seedlings, Rivera-Mendes et al., conducted experiments with four water conditions for 60 days: moderate deficit, field capacity, and partial and continuous flooding [41]. Seedlings under permanent flooding had a 22% reduction in biomass when compared to that of the control, due to the higher rates of leaf respiration and limitations in the absorption and transport of macronutrients. Plants under partial waterlogging showed growth similar to that observed under optimal soil moisture conditions.

In addition to the relationship with climatic variables, the correlation of fatal yellow with soil fertility and plant nutrition was studied. The disease had a positive correlation with Fe, K and Mn found in the soil. Thus, there was an increased incidence in places with higher levels of these minerals in the soil. This positive relationship may have occurred due to deficiency in the absorption of the roots or the imbalance of these cations when compared to other cations in the soil. The nutrients P, Ca, Mg and B had a significant negative correlation, i.e., the disease decreased with increases in these elements in the soil. Seeking to understand the role of other nutrients in soil with FY (FY?), Silveira et al., carried out an experiment with the omission of the macronutrients nitrogen, phosphorus, potassium, calcium, magnesium and sulfur and the micronutrients boron, iron, manganese, copper, zinc and molybdenum [42]. These authors observed an increase in symptoms when there was omission of micronutrients other than zinc and less incidence in treatments with omission of macronutrients other than Ca and S. Several studies on nutrition and fertilization have been carried out to verify the relationship between mineral elements and FY. Research has not verified the simultaneous effects of soil class, soil fertility and plant nutrition. When fertilization was studied, foliar analysis of the nutrients was not performed. Therefore, it is not possible to determine if the nutrients were absorbed or if their lack was associated with the disease. In studies analyzing the mineral nutrients in the plant, soil analysis was not carried out to determine whether deficiencies in a certain nutrient in plants was caused by low concentrations in the soil or due to inadequate absorption, including that possibly due to excess water in the soil, which would cause anaerobic conditions for the roots. Therefore, studying plant nutrition and soil fertility simultaneously is essential to understand the plant's physiological processes.

As for nutrition, palm trees showed deficiency in the nutrients N, P, Ca, Mg, S, Zn and Cu and exhibited excesses of K and B. According to Guzmán, AUDPC had significant negative correlations with the nutrients N, Ca, Si, B and Fe and a positive correlation with K. The minerals P, Ca and Mg also had low levels in the soil, which is a possible explanation for their deficiency in plants. The N, S, Si, Fe, and Mn were present in adequate quantities in the soil. However, in the sampled plants, they were below the nutritional levels suitable for the crop. Among these elements, N and S are structural constituents of plant cell components, including chlorophyll, amino acids, nucleic acids, coenzyme A, S-adenosylmethionine, biotin, vitamin B1 and pantothenic acid. Therefore, their deficiencies inhibit plant growth, causing chlorosis and yellowing of the leaves. Iron deficiency is common when water saturation occurs in the soil because this causes anaerobiosis in the root system. Its characteristic symptoms are chlorosis between the ribs. The relationship between iron and FY was investigated by Viégas et al., with four treatments of different doses of ferrous sulfate [43]. Afterwards, the iron contents in the oil palm leaves were measured, they caused no reduction or increase in symptoms in the analyzed plants.

Silicon, on the other hand, is not an essential nutrient for many plant families. However, some species accumulate substantial amounts of this element in their tissues and exhibit enhanced growth, fertility and stress resistance when supplied with adequate amounts of this element. Thus, plants deficient in silicon are more susceptible to tipping and fungal infection. Freitas et al., determined the role of silicon in reducing the severity of yellow Sigatoka (Mycosphaerella musicola) in banana trees grown in nutrient solution. The treatments had five concentrations of silicic acid (H_4SiO_4):

0. 0.5, 1.0, 1.8 and 3.6 mmol/L. The AUDPC for plants in the 3.05 mmol/L $H4SiO4$ treatment was 49.27% lower than that for plants without supplementary $H4SiO4$. In contrast, plants grown in a 3.6 mmol/L solution of $H4SiO4$ showed 23.53% more Si content in the leaves than plants grown without $H4SiO4$ supplementation. Thus, Si could be used in disease management.

Approximately 65% of oil palm trees had excess K and Mg deficiency. Potassium is the second most required nutrient in plants, and in the soil solution, it appears as the ion $K+$, which is absorbed in the roots of plants. In addition, $K+$ permeates plasma membranes, making it easily absorbed and transported in xylem and phloem. However, at high concentrations of this nutrient, the absorption of $Ca2+$ and $Mg2+$ is reduced by competitive inhibition. Mg deficiency induced by excess K in fertilization is common in crops such as banana and coffee, as they require large amounts of K. Mg is present in the chlorophyll molecule. In addition, it participates in enzymatic activation, acting as a cofactor for phosphorylative enzymes by forming a bridge between ATP or ADP pyrophosphate and the enzyme molecule. Therefore, deficiency in this element is characterized by yellowing of the older leaves.

In the soil classes PACal md/arg, PACd md/arg and PACal + PACd md/arg, with the highest amount of clay, there was a higher frequency of plots with FY incidence. The textural class is determined by the particle size distribution and affects other physical properties, such as drainage and water retention, aeration and soil consistency. The texture and structure of the soil define the surface area and porosity, which are the main factors associated with the storage and availability of water in the soil and the drainage of water from the surface to deep layers of the soil profile [44]. Silveira et al., analyzed the physical and chemical properties of 19 pedological profiles distributed in different plots of crop plantations. The authors classified the soil as a medium-textured yellow Latosol and showed the presence of compaction (1.365 kg/m^3 to 1619 kg/m^3) at depths of 30 and 60 cm. Thus, saturation of the soil in the superficial layer occurred during the period of greatest rainfall in the year, causing oxygen deficiency in the superficial layer of the soil. These conditions can be harmful to oil palm, causing root rot, due to its root system being dominantly distributed horizontally close to the soil surface. Regarding water in the soil and drainage, Bernardes measured the water potential in the soil with a tensiometer with low soil aeration with values between 0 bar and 0.075 bar, while 0.075 bar and 0.30 bar are more suitable for the plant [45]. Thus, areas at risk of flooding, which is related to the soil class, can cause anoxia of the oil palm's superficial roots, triggering nutritional deficiency and weakening the plant's resistance, making them more susceptible to infections by pathogens.

The proteomes showed proteins related to both biotic and abiotic stresses, especially in response to hypoxia, flooding and oxidative stress, upregulated in FY plants. Considering the studies and the lack of consensus regarding the origin of this disease, the identification and quantification, as well as understanding the implications of the differential abundances of these proteins in the metabolism of the plants affected by FY, becomes fundamental to suggest this origin.

Reactive oxygen species (ROS) are related to the regulation of signaling pathways and initial responses that occur to environmental stresses of biotic or abiotic origin [46, 47]. Antioxidant system-related proteins like peroxidase 2 and 1-Cys peroxiredoxin (Fragment) were upregulated in plants with FY and Asy plants with little difference in their abundance. In this case, oxidative stress can be an indication of stress from soil flooding in the area of plant cultivation.

In the data, primary metabolism-related proteins involved in energy production were also identified as having little difference in abundance between conditions. Despite this, they were overall more accumulated in Asy plants.

Additionally, with regard to energy metabolism-related proteins, alcohol dehydrogenases were identified in the roots of FY and Asy plants. Alcohol dehydrogenases are involved in alcohol fermentation during hypoxia or anoxia. There is a decrease in energy production through oxidative phosphorylation. Fermentative metabolism promotes energy compensation through recycling of $NAD+$ to the glycolytic pathway. Increases in the glycolytic pathway and alcohol fermentation-related proteins has been identified as a key response of plants to flooded soils [48-50]. Adaptive mechanisms to handle oxygen deprivation include synthesis induction of a set of approximately 20 proteins known as anaerobic stress proteins (ANPs), which in addition to alcohol dehydrogenase, also includes carbohydrate metabolism-related proteins, such as those identified in this study. In this context, the results indicated that the analyzed plants were likely subject to hypoxic conditions, which were probably caused by soil flooding in the crop area before or during the sampling period of the roots analyzed in this study.

It is important to highlight the identification of flooding stress-related proteins, such as polygalacturonase, that was identified with no significant differential abundance comparing the proteomes of plants with or without FY (Dataset Sx) [51]. However it was downregulated in plants with FY. This protein acts on the degradation of the cell wall, which leads to the formation of lysigenous aerenchyma., Aerenchyma channels formation can also occur by ROS-induced programmed cell death, which in the roots can provide flooding tolerance by improving oxygen and nutrient uptake under hypoxic conditions. In maize submitted to flooding stress, the increase in polygalacturonase expression and formation of aerenchyma is related to an adaptive response and allows greater toleranceto flooding [52]. Therefore, the high levels of these proteins in plants with and without symptoms may be related to the occurrence of flooding in these environments. Although not significant, the higher levels of polygalacturonase in asymptomatic plants may be related to their possible tolerance to flooding.

Among the proteins upregulated in plants with FY are calcineurin B-like protein 10 (Q7FRS8) and steroid 5-alpha-reductase DET2 (Q2QDF6), which have been attributed to the response to starvation. Calcineurin B-like protein 10 acts as a Ca+ sensor in cell signaling in response to environmental stimuli such as salinity, osmotic stress and nutrient deprivation, among others, including response to pathogens. This protein is involved in the activation of cellular responses from its binding and activation of serine/threonine-protein kinase. Serine/threonine-protein kinase was also upregulated in plants with FY. Caltractin (P41210) has also been identified in plants with FY, being known as Ca-binding and signaling proteins in response to environmental stimuli [53]. Regarding steroid 5-alpha-reductase DET2, this protein acts in the biosynthesis of steroids like brassinosteroid, which is involved in the response to many environmental stresses [54]. However, the sequence identified in this dataset was assigned only to starvation. The higher levels of these proteins in plants with FY also suggests the weakness of these plants, including nutritional deficiency and infection by pathogens due to their likely susceptibility.

Under nutrient deficiency conditions, plants need to increase their efficiency in nutrient storage and transport. Although the nodulin-like protein is well known for its characterization in plant roots in symbiotic association with microorganisms, studies have highlighted the importance of this protein in the transport of nutrients and other molecules to favor the growth and development of even non-nodulating plants [55-56]. Considering the response to abiotic stimuli, the nodulin-related protein 1 (Q9ZQ80) identified in this dataset was attributed to the osmotic stress and response to cold and heat. However, the possibility that it is also contributing to the increase in nutrient transport efficiency in FY plants cannot be excluded.

The translationally-controlled tumor protein (TCTP, Q9ZSW9) was attributed only to the term water deprivation. On the other hand, in addition to being involved in water transport, studies have shown that the increase in levels of this protein is related to photosynthesis and fatty acid metabolism in response to environmental stresses [57]. TCTPs have also been considered important in the defense response to fungal infections in plants [58]. Therefore, the higher levels of these proteins in plants with FY may include responses to several environmental stresses, including the increase in infections by pathogens.

Defense response proteins were also identified in plants with and without FY symptoms. In symptomatic plants, five fungal response proteins were upregulated, while four were downregulated. Regarding proteins in response to bacteria, only two were upregulated in plants with FY, while three were downregulated. The little difference between the abundance of fungal or bacterial response proteins observed in the protein profiles of healthy and affected plants does not allow us to deduce that fungal or bacterial infection is the major factor by which plants develop FY symptoms. Furthermore, asymptomatic plants showed higher levels of proteins related to the response to nematodes and insects. In general, asymptomatic plants seem to respond better to different types of environmental stresses, which should be attributed to their healthy state or the difference in genotypes that should be studied in the future.

We cannot yet assure why some genotypes have not yet developed the symptoms of FY. On the other hand, considering the results obtained in the analysis of climate variables, soil classification and fertility, plant nutrition and the protein profiles of plants with FY symptoms, it is possible to suggest the flooding as one of the factors for the deficiency in the nutrients N, P, Ca, Mg, S, Zn and Cu in the observed plants, which may be associated with the greater susceptibility of these plants.

5. Conclusion

Therefore, from all the analyses carried out, the edaphoclimatic conditions related to the accumulation of water in certain soil classes were related to the FY of the oil palm, which was related to the fertility and nutrition of the plant. This hypothesis was also confirmed by proteome analysis, which identified the environmental stress conditions of the roots in plants with symptoms. Under these conditions, several pathogens, both on the ground and in the aerial parts, may be opportunistic and associated with the symptoms observed, but they are not the primary cause of the disease. Therefore, a better understanding of the abiotic factors associated with this disease is essential to understand its etiology and in choosing locations and soils appropriate for the crop and which management strategies should be used. Thus, considering the abiotic origin of the disease caused by temporary flooding in oil palm cultivation sites, it is recommended that before planting that the soil pedology be surveyed, that soils less subject to flooding and good drainage are chosen, and that the soil is unpacked. Planting in ridges is also recommended to prevent root anoxia. In addition, periodic analyses of plant nutrition should be performed to maintain good soil fertility to avoid nutritional deficiencies and imbalances. In this way, planting can help plants adapt to the biome in the environment, achieving financial and social sustainability for forest populations.

Acknowledgments

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