Preliminary Analysis of Lignocellulose Content and Monolignol Composition of Oil Palm Trunk from Two Different Genetic Backgrounds

Ho Li Sim,* Tan Bee Aik, Normahnani Md Noh, Siti Sarah A. Talib, Nalisha Ithnin, Leona Daniela Jeffery Daim, Tony Ooi Eng Keong, and Hirzun Mohd Yusof

A preliminary investigation of chemical and lignin composition was conducted from samples of oil palm trunk of two different genetic backgrounds. A significant difference in percent (%) of standing palms was noted for the two different genetic backgrounds after 24 years of planting. Given that these palms were planted in neighboring fields, the objective of this preliminary study was to compare the chemical composition, as well as the lignin composition of the two palm varieties. When comparing the two populations, significant differences were observed in the structural carbohydrate composition and the lignin composition. This research constitutes the first reporting on the pyrolysis-gas chromatography-mass spectrometer analysis of oil palm trunk lignin composition.

Keywords: Monolignol composition; Oil palm trunk; Py-GCMS; Chemical composition; Structural carbohydrate

*Contact information: Sime Darby Technology Centre, 1st Floor Block B, UPM-MTDC Technology Centre III, UPM, 43400, Serdang, Selangor, Malaysia; *Corresponding author: ho.li.sim@simedarby.com*

INTRODUCTION

Since it was first introduced to Malaysia in the early 1870's, the oil palm has now been planted across 4.49 million hectares in Malaysia. This accounts for a production of about 17 million tonnes of palm oil and 2 million tonnes of palm kernel oil, making the nation one of the largest producers and exporters of palm oil in the world. Malaysia produces 11% of the world's oils and fats and 27% of the export trade of oils and fats.

Despite being a major crop cultivated in Malaysia, little is known about the lignocellulose composition of oil palm trees. The dry matter of the plant is composed of lignocellulose, which is referred to as lignocellulosic biomass. It is the most abundantly available raw material in the world and has potential for application in biofuel production. Lignocelluloses are composed of carbohydrate polymers, *i.e.*, cellulose and hemicelluloses (the structural carbohydrates), and aromatic polymers, *i.e.*, lignin, which are tightly bound together (Sjöström 1993).

The deposition of lignin in many parts of a plant provides structural strength. The major component in the cell wall of the xylem is impermeable to water, which is a characteristic that allows the transport of water within the plant. In the context of plant pathology, lignin can provide a barrier to microbial attack, and hence it has important implications for the oil palm industry. Basal stem rot (BSR) disease is widely recognized as an important condition that the industry has been facing. The pathogenesis of BSR, which is caused by the *Ganoderma boninense* white rot fungi, is generally believed to cause

the degradation of lignin in oil palm trees. White rot fungi are from a class of organisms that are capable of mineralizing lignin efficiently (Kirk and Cullen 1998). Different types of white rot fungi degrade lignin and carbohydrates in woody tissue at different rates and preferences. Some remove lignin more readily than carbohydrates, relative to the ratio of these two classes of biopolymers. The lignin component has been described as the rate-limiting factor in the biodegradation process (D'Souza *et al*. 1999). In addition, Paterson (2007) mentioned that lignin biodegradation is possibly the most influential part of the disease process. Guaiacyl (G) units of lignin are known to be more resistant to degradation than syringyl (S) units (Hatakka 2005). Studies show that the physiological condition of the plant is as important as the enzymes expressed by the fungi. Many studies have focused on the chemical composition of the oil palm trunk for its potential application in pulp processes, alternative sources of compressed wood, and/or other economic utilization (Khalil *et al*. 2007; Sitti Fatimah *et al*. 2012; Sulaiman *et al*. 2012; Lai and Idris 2013).

In 2013, while breeders were carrying out a standard census for progeny trials, two neighboring trials were found to have significantly different numbers of standing palms after 24 years. These progeny trials, namely the Dumpy AVROS (PT93) and Tanzanian (PT95), were established in 1989 by the Klanang Bharu Division, Sime Darby Plantation, Selangor, Malaysia. In 2013, at 24 years of age, the percentage of standing palms was 51.38% and 88.88% for the PT93 and PT95 backgrounds, respectively. Based on this observation, it was of potential interest to find out the contributing factors that influence the difference in the number of standing palms, given that these palms were grown in the same environment. The collapse of the palms was possibly attributable to the weakening in physical structure of the oil palm trunk. Therefore, it is postulated that: (i) the natural physical strength can be influenced by genetics and/or agronomy practices (Stackpole *et al*. 2011), or (ii) it may be caused by disease, such as BSR (Ariffin *et al*. 2000). In both cases, the condition of the physical structure is reflected in the composition of the trunks (Winandy and Rowell 2005). Hence, the objective of this study is to conduct a preliminary comparison of the chemical composition of oil palm trunks from two different genetic backgrounds.

EXPERIMENTAL

Selection of Oil Palm Trees

Oil palm trees of Tanzania (PT95) and Dumpy AVROS (PT93) lines, planted in neighboring fields located in Klanang Bharu Division, Sime Darby Plantation, Selangor, Malaysia, were selected for use in this study. For each background, two predetermined healthy palms were selected for each trial.

Samples Extraction and Preparation

Tissue samples from the oil palm trunks were harvested from approximately one foot above the ground. The samples were extracted using a motor drill at four points as shown by the cross configuration (⊗) in Fig. 2. Samples were extracted to the depth of approximately 20 to 24 cm radius. Samples from all points of the individual oil palm trunk were mixed, homogenized, and oven-dried to a constant weight at 60 °C. The dried tissues were then ground into fine particles and used for further analysis.

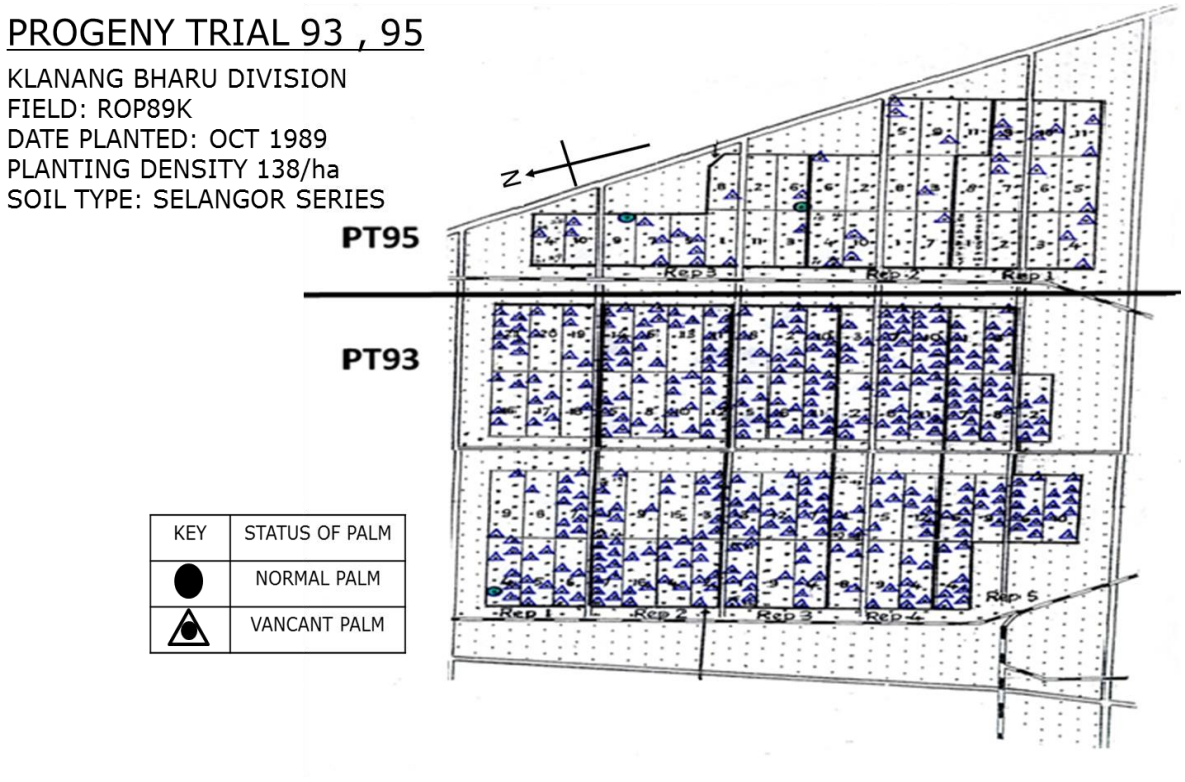

Fig. 1. Location map for progeny trials, PT93 and PT95. These two trials were neighboring to each other while the total number of standing palms was different after 24 years of planting

Analysis of Structural Carbohydrates

Three mL of 72% (v/v) sulphuric acid was added to 600 mg of sample, and the mixture was stirred for one min. Then, the mixture was incubated at 30 °C for 60 min and stirred at 5 to 10 min intervals to mix. Eighty-four mL of distilled water was then added into the mixture and mixed by inverting several times.

The preparation of the sugar recovery standard (SRS), which included glucose, xylose, arabinose, mannose, and rhamnose, was carried out using 1.0% of each individual sugar, which was added into 100 mL of water and 384 mL of 72% (v/v) sulphuric acid. The samples, SRS, and blank (without sugar) were then autoclaved at 121 °C for 60 min. The hydrolysates were cooled to room temperature and neutralized to a pH of 5 to 6 using calcium carbonate.

Neutralized hydrolysates were subjected to filtration using 0.2 µm polytetrafluoroethylene (PTFE) filter paper. A dilution was performed according to the calibration standards for the samples, SRS, and blank, followed by high performance liquid chromatography (HPLC) analysis using a HPX-87P column (Bio-Rad, USA). Twenty µL of each sample was injected at a flow rate of 0.6 mL min^{-1}, using water as mobile phase. The column temperature was set at 85 °C and the evaporative light scattering detector (ELSD) detector was set for a total run time of 35 min. Two technical replicates were used in this experiment.

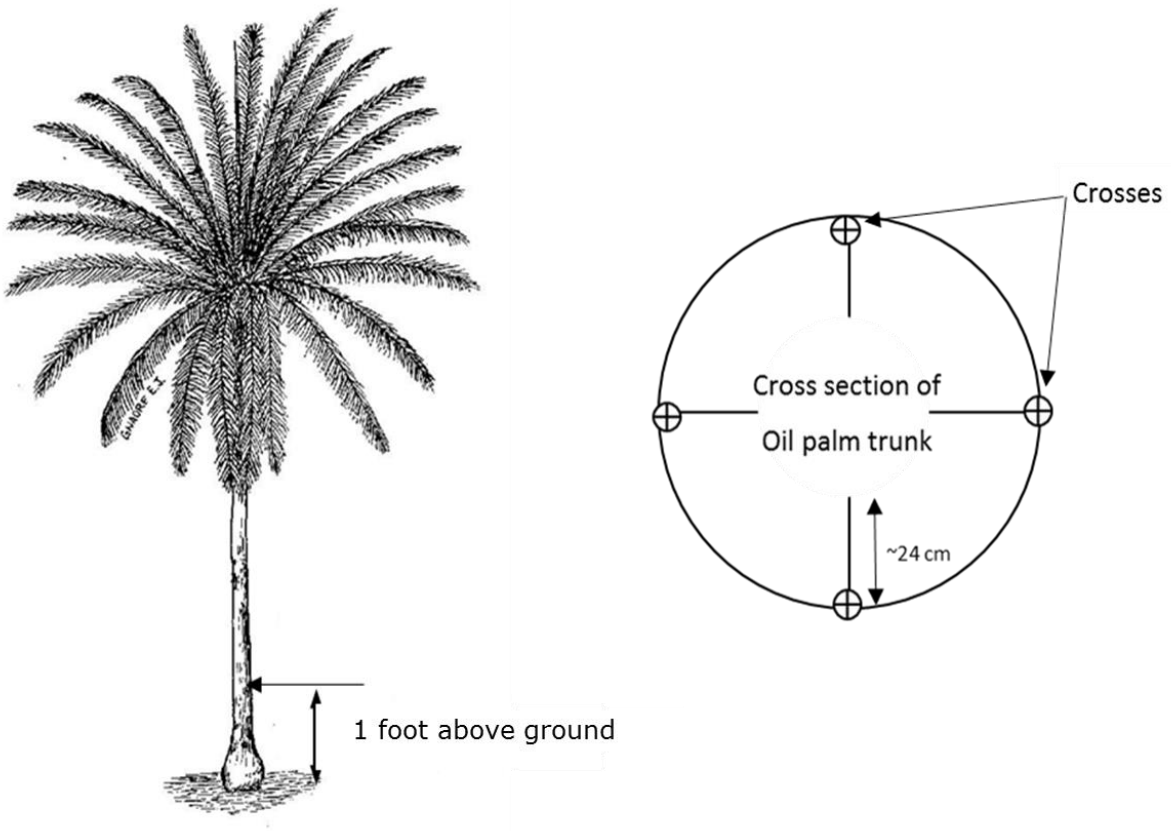

Fig. 2. Tissue samples from the oil palm trunks were extracted at approximately one foot above the ground at four points as shown by the crosses (illustration not presented to scale)

Lignin Content

The dried samples were sent to the Forest Research Institute Malaysia (FRIM), Selangor, Malaysia, for analysis of the lignin content using the TAPPI standard protocol, T222 om-02 "Acid-Insoluble Lignin in Wood and Pulp" (2011).

Pyrolysis-Gas Chromatography-Mass Spectrometer Analysis of Lignin Composition

Oven-dried samples were successively extracted using the Rencoret's Method (Rencoret *et al*. 2011). Briefly, the dried samples were extracted with acetone in a Soxhlet apparatus (Supelco, USA) for 8 h and with hot water for 3 h at 100 °C. The extracted samples were oven-dried to a constant weight and then subjected to pyrolysis-gas chromatography-mass spectrometer (Py-GCMS) analysis. The Py-GCMS was performed with a 2020 microfurnace pyrolyzer (Frontier Laboratories, Fukushima, Japan) connected to an Agilent 7890A GC-MS system equipped with a DB-1701 fused-silica capillary column (30 m x 0.25 mm; 0.25 µm film thickness) and an Agilent MSD5975 triple Axis Detector (Agilent Technologies, USA). Individual samples were weighed, and pyrolysis was performed at 500 °C. The GC over temperature was programmed from 450 °C (4 min) to 260 °C at a rate of 4 °C min^{-1} for 20 min. Helium was used as the carrier gas (with a flow of 1 mL min^{-1}).

Compounds were identified by comparing their mass spectrum data with corresponding values in the Wiley and National Institute of Standards and Technology (NIST) libraries, as well as those reported in the literature (Rencoret *et al*. 2011). The peak molar area was calculated for the lignin degradation products, the total calculated area was normalized, and the results of calculations were expressed as percentages.

RESULTS AND DISCUSSION

Chemical Composition of Oil Palm Trunk

Overall, the cellulose and hemicellulose (mainly xylose) content for the oil palm trunks of PT95 and PT93 are shown in Table 1. The structural carbohydrate composition of the oil palm trunks of Tanzanian (PT95) and Dumpy AVROS (PT93) were analyzed. Figure 3 shows the individual sugar composition from the PT95 and PT93 trunks. Based on the two biological replicate analyses, the composition of the PT95 and PT93 for glucose, galactose, xylose, and arabinose were 37.48% and 44.16%, 25.33% and 25.67%, 6.79% and 7.25%, and 4.74% and 4.26%, respectively (Fig. 3).

Table 1. Cellulose and Hemicellulose Content of PT95 and PT93

	PT95 (%)	PT93 (%)
Cellulose	37.48	44.16
Hemicellulose*	36.86	37.18

*Hemicellulose is referred as the sum of xylose, galactose, and arabinose

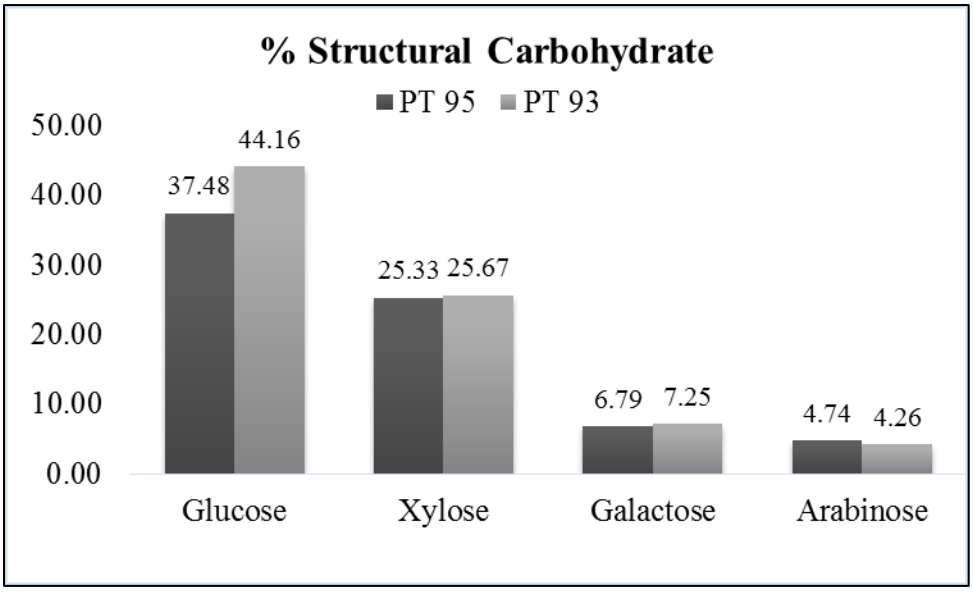

Fig. 3. Composition of the structural carbohydrates for PT93 and PT95

On the other hand, when compared to PT95, a consistent trend was observed. The PT93 exhibited a higher ratio of glucose, galactose, and xylose to arabinose (Fig. 4). In research by Dahal and colleagues, it was mentioned that the inhibition of pathogens could occur by limiting the supply of sugar. In his study, sugar limitation was associated with a suppression of anolase in disease-resistant tomato (Dahal *et al*. 2010). Although the underlying reason for the sugar content is not clear, it was observed that the PT93 with a smaller number of standing palms were shown to have a higher overall sugar content compared to PT95.

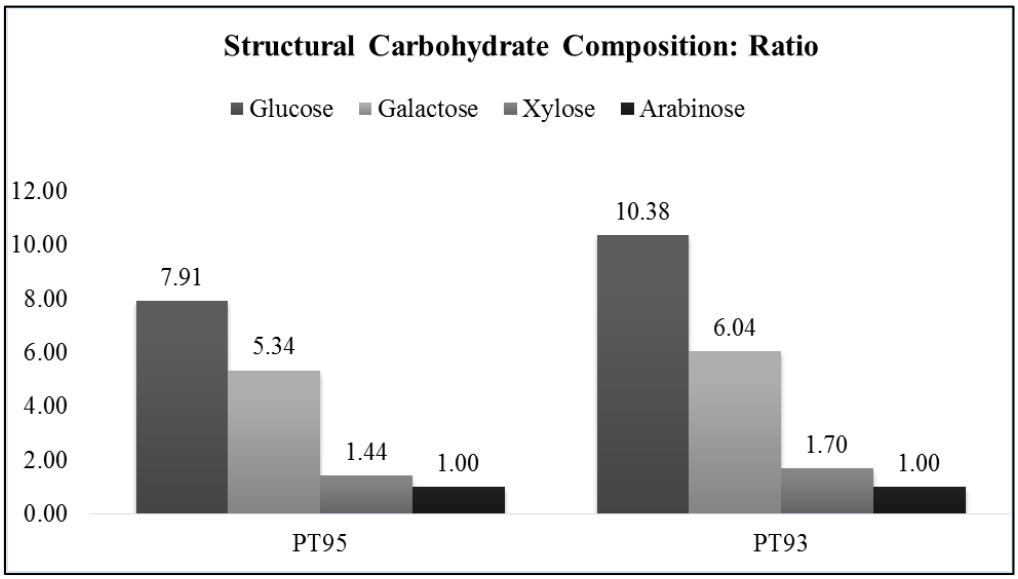

Fig. 4. The ratio for structural carbohydrate composition for PT93 and PT95. The ratio was computed against the carbohydrate type with the smallest quantity within the sample

The acid-insoluble lignin and the monolignol composition analysis were done on the same set of samples. For the acid insoluble lignin method, lignin was defined as wood constituent that is insoluble in 72% sulfuric acid. It was found that the acid insoluble lignin content of PT95 and PT93 was 24.8% and 25.0%, respectively. These results were in agreement with the work done by Abdul Khalil and colleagues, where 24.51% of acid insoluble lignin was recorded in the oil palm trunk sample (Abdul Khalil *et al*. 2008). There were no apparent differences observed in the acid insoluble lignin content when comparing PT95 to PT93 samples.

Characterization of Pyrolysis Products of Oil Palm Trunk

Lignin is a complex of phenolic polymers that provides an embedding material for cellulosic polymers in not only the secondary cell walls, but also in the middle lamellae between adjacent cell walls (Plomion *et al*. 2001). Lignin not only provides mechanical strength to a plant, improving its physical and chemical properties, but also serves as a barrier against the invasion of pests and pathogens (Bhuiyan *et al*. 2009). There is variation in the amount of lignin within a plant and within species. In addition, the composition of lignin is highly variable. In general, there are three monolignol monomers which are methylated to various degrees, *i.e.*, p-coumaryl alcohol, coniferyl alcohol, and sinapyl. When incorporated into lignin these monomers form p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively. Softwoods typically contain a majority of G-type lignin,

while hardwoods contain a combination of G- and S-type lignin. This is characterized by the ratio S/G, which varies among species (Río Andrade *et al*. 2011).

Pyrolysis is a relatively simple thermochemical method in which lignin is broken down into lower molecular weight compounds in the absence of oxygen. Though it is a semi-quantitative method, Py-GCMS has been used in a number of analyses on monolignol composition of plant materials (Ralph and Hatfield 1991; Meier *et al*. 2005; Rencoret *et al*. 2011; Ross and Mazza 2011; Ohra-Aho *et al.* 2013). Similar profiles were observed from both polygrams obtained for the PT95 and PT93 samples (Fig. 5).

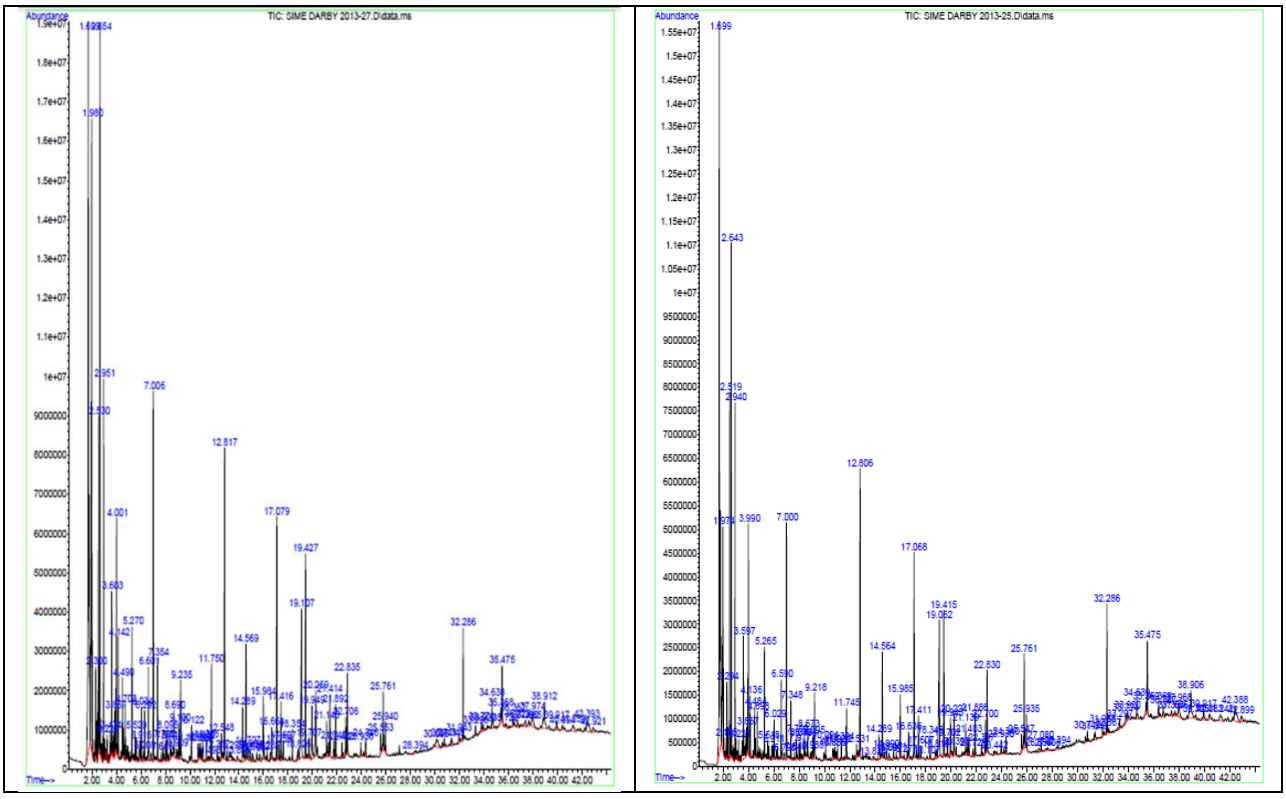

Fig. 5. Py-GCMS Chromatogram of the PT95 (left) and PT93 (right) samples. Similar profiles for both samples were observed.

The identities and relative molar abundance of lignin-derived compounds, which fulfilled greater than 80% similarity (Lou *et al*. 2010) against the NIST library are listed in Table 2. The values in Table 2 are referred to as the percent of relative areas in each lignin-derived compound per milligram of sample. Lignin-H sum, lignin-G sum, and lignin-S sum represented the total amount of identified lignin products from p-hydrophenyl, guaiacyl, and syringyl lignin units, respectively. There were a total of 23 lignin-derived compounds identified from the Py-GCMS analysis for samples of oil palm trunk, of which 17 lignin-derived compounds were detected in samples of both PT95 and PT93. There were five distinct lignin-derived compounds detected in PT95 and one distinct lignin-derived compound detected in PT93, as indicated in Table 2.

Lignin composition depends on the genetic origin of the species of plant (Ross and Mazza 2011). The amount of lignin varies between species and within individual plant tissues. Usually, monocotyledonous plants, such as wheat, barley, and oat contain products of guaiacyl (Lg-G), syringyl (Lg-S), and p-hydroxyphenyl (Lg-H) units in their lignin

polymers, and usually have much lower concentrations of Lg-H (Ross and Mazza 2011). On the other hand, plants that are dicotyledonous usually contain products of Lg-G and Lg-S. In the samples of oil palm trunk, the most abundant lignin pyrolysis products were found to be originated from Lg-S. There were almost equal concentrations of Lg-G and Lg-H products detected in the samples obtained from PT95. However, the samples from PT93 contained a higher concentration of Lg-G products over products of Lg-H (Table 2). The observation where there are relatively higher Lg-H products detected in samples of oil palm trunk, was found to be different from observations of other monocotyledonous plant species. In comparison, other plants exhibited considerably lower total products of Lg-H when compared to Lg-G, and Lg-S (Martínez *et al*. 2008; Ragauskas ; Rencoret *et al*. 2011; Ross and Mazza 2011). Sattler and Deanna mentioned that "defense" lignin was often shown to have elevated levels of Lg-H-subunits, for which the deposition of Lg-H was induced as part of cell wall components upon exposure to stresses as compared to structural lignin (Sattler and Deanna 2013). The palms in the current study had experienced a long standing time in open, monoculture fields and were exposed to both biotic and abiotic stresses which may have induced deposition of subunit of H-lignin in a slow but progressive way.

It was observed that PT95, with a higher number of standing palms after 24 years of planting, exhibited a lower Lg-G and a higher Lg-S and S/G ratio when compared with PT93 (Table 2). While most of the studies focused on modified lignin related genes caused a reduction or an increase of total lignin content, there are no general trends drawn in the relationship between total lignin content and disease resistance. Researchers had shown that suppression or down-regulation or silencing of lignin or monolignol related genes, which led to reduced content of lignin, compromised disease resistance against fungal pathogens and pests in a wide range of plants tested (Bhuiyan *et al*. 2009; Xu *et al*. 2011). Others have shown that plants with reduced lignin content exhibited no changes of susceptibility (Sattler and Funnell-Harris 2013). In some cases, for example, Funnel and team showed that genetically modified sorghum with reduced lignin content were found with increased resistance to *Fusarium* spp., including *F. moniliforme*. (Funnell and Pedersen 2006). Selective lignin down-regulation which caused reduced-lignin content was shown to indirectly result in constitutive defense response expression in alfalfa (Gallego-Giraldo *et al*. 2011).

In discussions on total lignin content, it was noticed that suppression of lignin synthesis pathway genes, ferulic acid 5-hydroxylase (*F5H)* or caffeic acid *O*-methyltransferase (*CAOMT)*, was shown to be relating to a reduction in the S/G ratio with little effect on the total lignin content of the plant (Chen *et al*. 2006; Li *et al*. 2008). In a similar study done on leaf tissues of wheat, the silencing of another lignin pathway gene *TmCAOMT* was found to efficiently enhance the fungal penetration depth into the plant tissues, indicating that S/G ratio is an important factor for pathogen penetration. Similarly, a *spi*-2 transformed-line of tobacco which displayed similar lignin level to the control lines was shown to have altered lignin monomeric composition and structure. The transformed line, exhibited increased susceptibility to the pathogenic oomycetes, but on the other hand, increased the ability of the mutant lines in suppressing growth of the pathogenic bacterium (Elfstrand *et al*. 2002).

Table 2: Identification and Relative Abundance (%/mg) of Lignin Related Pyro-Lysates for PT95 and PT93

No.	Lignin related pyrolysate	Monolignol origin	PT95		PT93	
			RT	Relative abundance	RT	Relative abundance
1	Phenol	H	7.000	2.47	7.006	2.52
2	Phenol, 2-methoxy (guaiacol)	G	7.348	0.53	7.354	0.84
3	Phenol, 2-methyl-	H	7.792	0.44	7.797	0.40
4	Phenol, 4-methyl-	H	8.432	0.64	8.443	0.62
5	Phenol, 2-methoxy-4-methyl-	G	9.095	0.31	9.100	0.33
6	Phenol, 2,3-dimethyl	H	9.948	0.11	-	0.00
7	Phenol, 3,5-dimethyl	H	-	0.00	9.948	0.17
8	Phenol, 4-ethyl-2-methoxy (4-ethyl guaiacol)	G	10.656	0.31	10.656	0.32
9	2-methoxy-4-vinylphenol (4-vinylguaiacol)	G	11.745	1.26	11.750	1.44
10	Eugenol	G	-	0.00	12.217	0.18
11	Phenol, 2,6-dimethoxy (syringol)	S	12.806	3.06	12.817	3.14
12	Phenol, 2-methoxy-4-(1-propenyl)-, (E)- (isoeugenol)	G	-	0.00	13.295	0.37
13	Phenol, 2-methoxy-4-(1-propenyl)-, (E)- (isoeugenol)	G	14.289	0.45	14.289	1.00
14	Vanillin	G	-	0.00	14.727	0.20
15	Ethanone, 1-(4-hydroxy-3-methoxyphenyl) (acetovanillone)	G	16.277	0.17	16.282	0.21
16	Phenol, 2,6-dimethoxy-4-(2-propenyl)- (methoxyeugenol)	S	17.411	0.51	17.416	0.51
17	Phenol, 2,6-dimethoxy-4-(2-propenyl)- (methoxyeugenol)	S	19.349	0.27	18.354	0.36
18	Phenol, 2,6-dimethoxy-4-(2-propenyl)- (methoxyeugenol)	S	19.415	1.63	19.427	1.54
19	Benzaldehyde, 4-hydroxy-3,5-dimethoxy (syringaldehyde)	S	19.943	0.52	19.949	0.57
20	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	G	21.403	0.32	21.414	0.78
21	2-propenal, 3-(4-hydroxy-3-methoxyphenyl)-	G	-	0.00	21.740	0.30
22	3,5-dimethoxy-4-hydroxycinnamaldehyde	S	25.935	0.46	25.940	0.30
23	Phenol, 4,4'-methylenebis[2,6-dimethoxy	S	35.475	2.00	35.475	1.22
	Lignin-H sum			3.66		3.71
	Lignin-G sum			3.35		5.97
	Lignin-S sum			8.45		7.64
	S/G ratio			2.52		1.28

The percent relative abundance per mg of sample for lignin derivatives was calculated using the average of two replicates. The ratio of the sum of the area under the peaks for the lignin derivatives per mg of sample was divided by the area of all peaks per mg of sample multiplied by 100%. All Lg-G- and Lg-S- derived peaks were used as an estimation of the S/G ratio

Another study was done on lignin content and lignin composition, where resistant and susceptible genotypes of *Populus tremuloides* had undergone artificial wounding and had been inoculated with pathogens. The results revealed that the Klason lignin was similar for both genotypes, but differences were observed for lignin monomeric composition when comparing the two genotypes. The susceptible genotype was found to accumulate higher level of lignin-subunit-H over the resistant genotype (Bucciarelli *et al*. 1998).

After 24 years of planting, there are many factors that contribute to the collapse of oil palms, and this could be attributed to physical force factors, *i.e*., strong wind, or a reduction in the disease-resistant capability of the plant. Nonetheless, the collapse of oil palms occurs because of the weakening of their mechanical properties. Variation in lignin content and composition within species is common. Guerra and team (2013) had suggested that a close relationship can be expected between the associated single nucleotide polymorphisms (SNPs) and the causative polymorphisms underlying the genetic variation of lignocellulosic traits based on their work done on black poplar. In addition to that, quantitative trait loci (QTL) analyses for lignin-related traits have shown that at close to 40 genomics regions are involved in maize variation of lignin content (Barrière *et al*. 2007). In another work done on *Eucalyptus urophylla*, SNPs that showed significant association with S/G ratio were identified (Denis *et al*. 2013). Although it is not clear whether the genetic variations are the "cause" for the differences as in nature, or the "cause" that causes the differences as in induced responses (upon exposure to biotic and abiotic stresses), nonetheless these evidences indicated the complexity of the regulation of the lignin-related gene. The reported results also indicated possible effects or control of genetic variation on physical strength, which may be direct or indirectly attribute to disease resistance ability of plant. While differences were observed in structural carbohydrate and Lig-G and S/G ratio comparing the two groups of oil palms, the case in oil palm has remained unclear to date. More work in this area is needed to explain the relationship between the lignocellulosic composition and physical strength of palm trees based on the results of this study.

CONCLUSIONS

1. Characterization of the chemical composition of oil palm trunk provided insight into the possible factors contributing to the mechanical properties of these palms. It was shown that oil palm trunk possess higher portion of Lg-H of its lignin monomer composition when compared to other monocotyledonous plant.
2. The structural carbohydrate, acid insoluble lignin, as well as lignin composition were analyzed for the samples obtained from PT95 and PT93. As a preliminary observation, oil palm trunk of PT95 with higher number of standing palms was found to have higher S/G ratio when compared to palms of PT93. Also, oil palm trunks of PT95 appeared to have lower content of structural carbohydrate when compared to PT93.
3. While the current article has reported the differences found between the two groups of oil palm, the current observations were done based on small number of biological replicates. Conclusive description on the relationship between lignocellulosic content

of oil palm and the physical strength which directly or indirectly related to resistance towards biotic and abiotic stress remains reserved. Same work should be repeated with larger scale within the same populations to confirm the observation.

4. Also, by utilizing the available information provided by the oil palm genome sequencing initiatives, genetics of lignocellulosic related components should be explored for oil palm. For example, the polymorphisms character of lignin pathway genes, which may be the underlying cause, should be examined. In addition to that, exploring the differential expression level of related genes of the two distinct group may be providing useful information in confirming the observation obtained in this study.

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