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Cluster analysis of polyisoprenoid in oil palm (*Elaeis guineensis*) leaves in different land-uses to find the possible cause of yield gap from planting materials

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Abstract. *Basyuni M, Wati R, Deni I, Tia AR, Slamet B, Siregar ES, Syahputra I. 2018. Cluster analysis of polyisoprenoid in oil palm (*Elaeis guineensis*) leaves in different land-uses to find the possible cause of yield gap from planting materials. Biodiversitas 19*: *1492-1501.* The distribution and occurrence of polyprenols and dolichols in the leaves of oil palm (*Elaeis guineensis* Jacq.) plantations in different land-uses in North Sumatra, Indonesia were analyzed using two-dimensional thin layer chromatography (2D-TLC). Eighty-one of oil palm leaves were sampled to represent twenty-seven sites of land-uses, namely paddy field (four locations), mangrove (three locations) in Lubuk Kertang, Langkat, North Sumatra. In addition, samples from four groups of smallholders in Stabat, Langkat, six sites in Bangun Bandar, Serdang Bedagai, and ten sites on the campus of Universitas Sumatera Utara (USU), North Sumatra were collected. In the leaves, only one type (type II) with respect to the distribution of polyisoprenoids was detected: having the presence of both polyprenols and dolichols. Either type I, having predominance of dolichols over polyprenols or type III, displaying dominating polyprenols over dolichols were not observed. Results also showed that chain-length distribution of ficaprenols (C_{50}-C_{60}) without longer polyprenols (C_{85}-C_{100}) and dolichols of C_{85}-C_{100} was detected in the paddy field, mangroves, and one site in USU campus. This polyisoprenoid profile was close to *dura* type of *Elaeis guineensis*. By contrast, the remaining land-uses had ficaprenols and longer polyprenols, and dolichols (C_{85}-C_{100}), which belong to *tenera* or *pisifera* type. To confirm this finding, a dendrogram was constructed. Cluster analysis demonstrated that twenty-seven sites of *E. guineensis* were grouped into appropriate types of *dura, pifiera,* and *tenera* accordingly, indicating that the existence of polyisoprenoids in *E. guineensis* was a chemotaxonomic marker. The finding of polyisoprenoid pattern of *E. guineensis* as *dura* type in mangrove and paddy field sites may reveal significant causes of yield gap in oil palm plantation from planting materials.

Keywords: Chemotaxonomic marker, mangrove, oil palm leaf, paddy field, smallholder

Abbreviations: 2D-TLC: two-dimensional thin layer chromatography, CPO: crude palm oil, Dol: dolichol, UPGMA: unweighted-pair group method with arithmetic mean, MVSP: multivariate statistical package, TL: total lipid, Pol: polyprenol

INTRODUCTION

Indonesia is currently the largest producer and exporter of palm oil in the world. The production of palm oil in Indonesia since 1964 has recorded a phenomenal rise from 157 k t to 35 m t in 2017 (Directorate General of Estate Crops 2017). To maintain its status as the world's largest palm oil producer, Indonesia has projected the figure of 40 m t by 2020 (McClanahan 2013). Sumatra and Kalimantan are the two islands that account for 96% of Indonesia's palm oil production. North Sumatra province is one of the centers of oil palm (*Elaeis guineensis* Jacq.) plantations in Indonesia. The North Sumatera area of oil palm plantation in 2017 was 1.47 m ha with CPO (crude palm oil) production of 5.7 mt (Statistics of Sumatera Utara 2017). Although Indonesia has large oil palm plantation area and becomes number one palm oil producer, Indonesian palm oil productivity is lower than that of Malaysia (Mukherjee and Sovacool 2014), suggesting an enormous yield gap (Woitties et al. 2017).

It is important to identify the significant causes of yield gap in oil palm plantations. Improvement of the oil palm from plant material can be done with two methods: conventional methods of palm oil breeding and modern methods of crop improvement with biotechnology. Conventional technology takes a long time, high cost, large area and lots of manpower. Therefore, a biotechnology approach with a focus on improving planting materials and increasing productivity of oil palm becomes the right choice as a basic capital for the establishment of a plantation system with high levels of productivity and efficiency (Wu et al 2009).

A number of molecular breeding studies have previously focused more on propagation of oil palm with tissue culture and its encoding genes (Low et al. 2008), somatic variation of embryogenesis for clonal propagation and controlling genes (Te-chato and Hilae 2007), geographic structure and genetic diversity (Singh et al.

2008) and the recent discovery of shell genes responsible for the shell thickness and size (Singh et al. 2013). Oil palm according to the thickness of the shell in the fruit, is divided into three types, namely *dura* (thick-shelled), *pisifera* (shell-less), and *tenera* (thin-shelled) - a hybrid between *dura* and *pisifera*, the source for commercial palm oil production worldwide (Basyuni et al. 2017a). Our previous research and other researches revealed that the molecular markers have not yet shown consistent results and there are still continuing studies to detect early types of oil palms before the fruit production phase (Ritter et al. 2016; Babu et al. 2017, 2018a). In addition, an alternative technique to separate the fruit types of oil palm (Arifiyanto et al. 2017) and other plants (Basyuni et al. 2016, 2017b,c,d; 2018a) by determination of polyisoprenoid compound has been reported using two-dimensional thin layer chromatography. These studies suggested that composition of polyisoprenoid in the leaves is reproducible for plant species and therefore is regarded as a chemotaxonomic species-definite marker (Rosalinka et al. 2002; Basyuni et al. 2016, 2017b; Arifiyanto et al. 2017). The present study was aimed to analyze the profile of polyprenols and dolichols in the leaves of oil palm (*E. guineensis*) plantations in different land-uses in North Sumatra, Indonesia and to obtain significant causes of yield gap in oil palm plantation from planting materials.

MATERIALS AND METHODS

Plant materials

Eighty-one of oil palm leaves were sampled to represent twenty-seven sites of different land-uses, namely paddy field (PF), consisting of four locations, PF1-PF4, mangrove forest with different salinity (MS) comprising three locations, MS 0.5%, 1%, and 2% in Lubuk Kertang Village, Langkat District, North Sumatra, Indonesia (Figure 1.A). In addition, ten sites on the campus of Universitas Sumatera Utara (USU), Indonesia, were also collected, i.e., Gate 4, Faculty of Engineering (FE), Gate 1, Gate 3, Faculty of Medicine (FM), University Library (UL), Civil Engineering (CvE), Faculty of Dentistry (FD), Chemical Engineering (CeE), and Faculty of Agriculture (FA) (Figure 1.B). Other leaves were sampled from four groups of smallholders, specifically oil palm *tenera* (OPT), oil palm abortion (OPA), oil palm PT Harapan Sawita (OPTHS), and oil palm marihat (OPM), in Stabat, Langkat, and six sites, i.e., Socfin Indonesia, Socfindo *dura* (SD), Socfindo wild type (SWT), Socfindo *tenera* (ST), Socfindo nursery (SN), and Socfindo *pisifera* (SP) in Bangun Bandar, Serdang Bedagai, North Sumatra (Figure 1.C-D). The sample site positions are described in Table 1. Leaves appearing first (age was approximately 2-4 weeks after opening) were regarded as samples. All Samples were collected when the average temperature of the environment was 25-28°C and humidity was 83-85%.

Chemicals

A mixture of dolichol (C_{90}-C_{105}) and polyprenol (C_{90}-C_{100}) standard compounds was used to detect the polyisoprenoids that were identified in this study, as previously described in Basyuni et al. (2018). The identification of the family corresponding to polyprenols or dolichols was performed in at least three independent experiments. Silica gel 60 TLC glass plates and reversed-phase silica RP-18 HPTLC glass plates were purchased from Merck. All of the other chemicals and solvents were of reagent grade (Merck).

Isolation of polyisoprenoid alcohols

The procedure of polyisoprenoid alcohols isolation was carried out as previously described in Basyuni et al. (2016, 2017a,c) and Arifiyanto et al. (2017). The samples of leaves were dried using an oven at 60-70°C for 1-2 days. The dried tissue was crushed into a fine powder using laboratory mills, then weighed 5 g each and immersed in chloroform/methanol (CM2: 1; v/v) solvent, then incubated in a water bath for 48 h. The supernatant was filtered using No. 2 filter paper (Advantec, Tokyo, Japan) then dried

Tabel 1. Description of sample locations in North Sumatra, Indonesia

Location	East longitudes (°)	North latitudes (°)
MS 0.5%	98.2749	04.0720
MS 1%	98.2776	04.0719
MS 2%	98.2812	04.0733
PF 1	98.2837	04.0740
PF 2	98.2868	04.0731
PF 3	98.2872	04.0766
PF 4	98.2782	04.0698
Gate 1	98.6451	03.5673
Gate 3	98.6408	03.5673
Gate 4	98.6383	03.5673
CeE	98.6421	03.5637
CvE	98.6424	03.5618
UL	98.6422	03.5612
FA	98.6401	03.5607
FD	98.6436	03.5658
FE	98.6408	03.5628
FM	98.6445	03.5667
SD	99.0378	03.3174
SP	99.0324	03.3204
ST	99.0348	03.3205
UOP	99.0408	03.3217
SWT	99.0242	03.3237
SN	99.0499	03.3397
OPT	98.4074	03.9757
OPA	98.4065	03.9970
OPTHS	98.2972	04.0581
OPM	98.4874	03.4757

Note: PF: Paddy field, MS: Mangrove salinity, CeE: Chemical Engineering, CvE: Civil Engineering, UL: University Library, FA: Faculty of Agriculture, FD: Faculty of Dentistry, FE: Faculty of Engineering, FM: Faculty of Medicine, SD: Socfindo *dura*, SP: Socfindo *tenera*, ST: Socfindo *tenera*, UOP: Unknown oil palm, SWT: Socfindo wild-type *tenera*, SN: Socfindo nursery, OPT: Small holder oil palm *tenera*, OPA: Small holder oil palm abortion, OPTHS: Oil palm PT Harapan Sawita, OPM: Small holder oil palm Marihat, TL: total lipid, PI: polyisoprenoid, Pol: polyprenol, Dol: dolichol, Socfindo: Socfin Indonesia

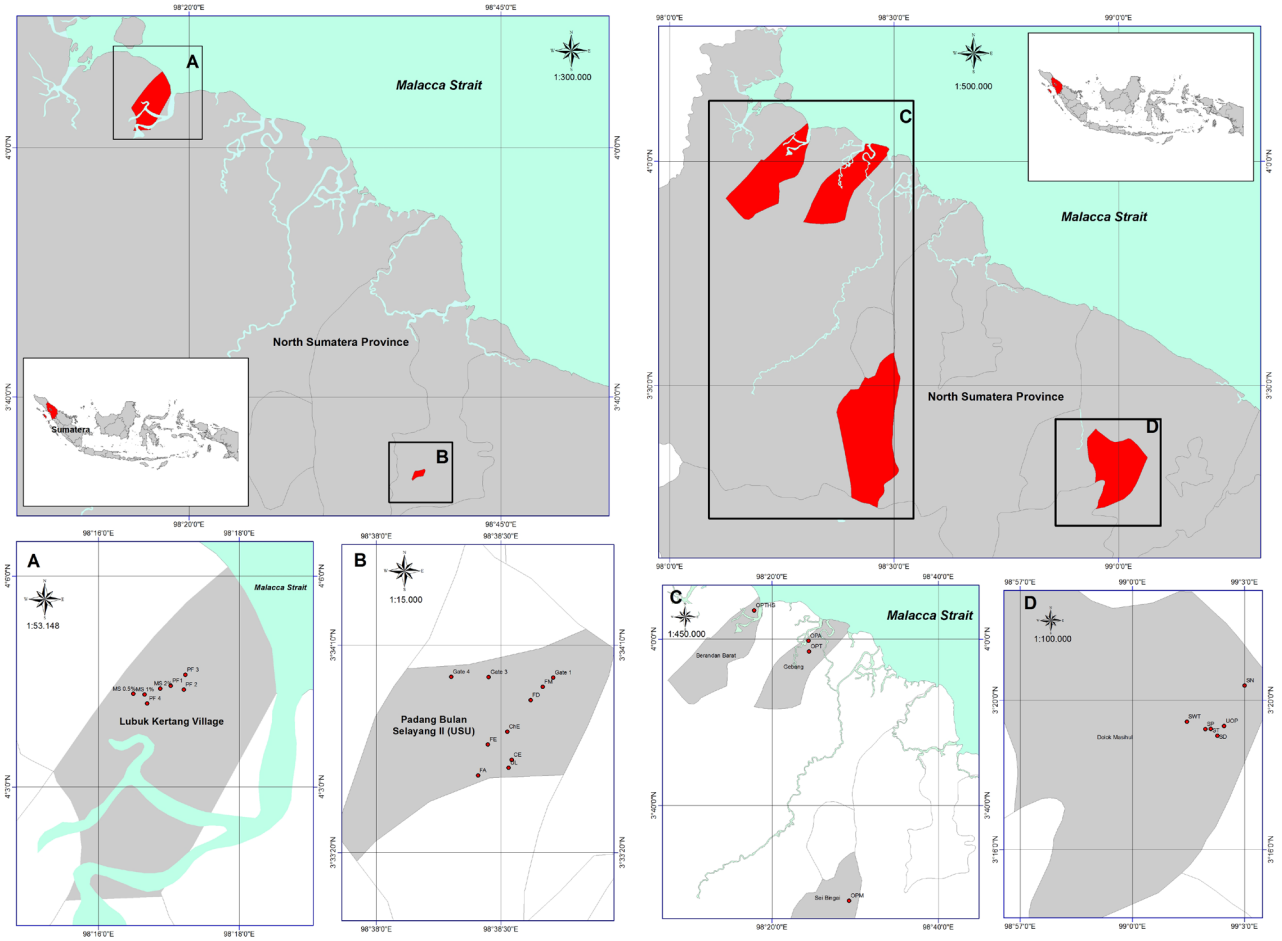

Figure 1. Map of study sites showing different land-uses of *E. guineensis* in North Sumatra, Indonesia

using rotary evaporator. The lipid extract of all samples was saponified at 65 °C for 24 h in 86% ethanol containing 2 M KOH. The unsaponifiable lipids of each sample were evaporated and re-dissolved in hexane. All the samples extract (approximately 100 mg) were applied to each TLC plate.

Analysis by two-dimensional thin layer chromatography (2D-TLC)

First-dimensional TLC was performed for 45-60 min on a silica gel glass plate (20 × 3 cm) with a solvent system of toluene-ethyl acetate (9: 1) as previously described in Basyuni et al. (2016; 2017a,d). Second-dimensional reversed-phase C-18 silica gel TLC was carried out with acetone as the solvent for approximately 45 min. The position of the separated polyisoprenoid alcohols being developed by 2D-TLC was identified and visualized with iodine vapor before scanning (Basyuni et al. 2017c). To determine whether the family corresponds to dolichols or polyprenols, in the case of the one family that was observed on 2D-TLC, a dolichol or polyprenol reference was added to the sample line of the first-dimensional TLC and developed with a solvent system, as previously reported in Basyuni et al. (2017d). The developed chromatographic images were obtained and digitally scanned with a Canon E-470 series printer. The polyisoprenoid family was determined by the comparison of mobility on TLC chromatogram with that of authentic standards of dolichol or polyprenol that were applied in the second-dimensional phase. The polyprenols and dolichols that were traced on the RP-18 HPTLC glass plates were semi-quantified using ImageJ ver. 1.46r (Schneider et al. 2012) with references of dolichol and polyprenol standards. The scan chromatogram was greyscale mode analyzed to get plot lines and label peaks. This area peak was copied and pasted to the program Microsoft Excel 2010 for quantification.

Cluster analysis

Phylogenetic tree analysis was done on selected subsets of leaf data consisting of 19 variables and 27 samples. All data were log (10) transformed as previously reported in Basyuni et al. (2018a). From these data, the dendrogram representing the leaf data was constructed by cluster analysis using the unweighted-pair group method with arithmetic mean (UPGMA) and MVSP (multivariate

statistical package) ver. 3.22 (Kovach Computing Service). Euclidean distance was chosen as the standard for cluster combination.

RESULTS AND DISCUSSION

Profile and distribution of polyisoprenoids in *E. guineensis*

The search for polyisoprenoids from the leaves of *E. guineensis* plantations in different land-uses conducted using 2D-TLC (Basyuni et al. 2016, 2018a) brought clear separation of polyprenols from dolichols with respect to the carbon chain length. Tables 1-2 summarize the occurrence and distribution of polyprenols and dolichols with the carbon-chain lengths in *E. guineensis* planted in mangroves area, paddy field, and campus of USU. The total lipids (TL) are denoted as a fraction of crude lipids gravimetrically assessed. The TL ranged from 54.1 to 78.7 mg g^{-1} dry weight. The quantity of PL varied among the locations. Only one type of profile group of polyprenols and dolichols in the leaves was detected, namely type-II with respect to the pattern of polyisoprenoids: having the occurrence of both polyprenols and dolichols (Basyuni et al. 2016, 2017d, 2018). The occurrence of both polyprenols and dolichols well agreed with previous findings on tropical plants (Tateyama et al. 1999; Basyuni et al. 2017d; Arifiyanto et al. 2017). The contents of Polyprenols were slightly higher than those of dolichols in most cases of mangrove areas and paddy fields (Tables 2-3). Similar results were also found in USU campus sites (Table 2).

The polyisoprenoid profile of oil palm in land-use of mangroves area and paddy field of Lubuk Kertang showed one family polyprenols and dolichos as well, except in PF1 which consisted of two families: ficaprenol (C_{50}-C_{60}) and longer polyprenol ($C8_5$-C_{100}). It has been suggested by our previous results (Basyuni et al. 2017b, 2018a) that the chain length of polyprenols varied from tissue to tissue even for the same species, and appeared to form distinct families. In this circumstance, polyprenols also occurred as one or two polyprenols families, depending on the locations. The occurrence of two polyprenols families was observed in PF1 (Figure 1.D, Table 2). Similar to this observation, two polyprenols families were also detected in the polyisoprenoids pattern in USU campus, except in Faculty of Agriculture (FA), only one family was also identified as *dura* (Table 2, Figures 2-4). By contrast, the remaining land-uses had ficaprenols and longer polyprenols (C_{85}-C_{100}), and dolichols (C_{85}-C_{100}), which belong to *tenera* or *pisifera* type. These results therefore support the opinion that there may be at least three different biosynthetic pathways responsible for the formation of ficaprenols (shorter polyprenols), longer-chain of polyprenols and dolichols in *E. guineensis* (Arifiyanto et al. 2017) as well in mangrove, coastal plants and tropical trees (Basyuni et al. 2016, 2017b,d, 2018).

The structural group of shorter carbon-chain of polyprenols/ficaprenols (C_{85}-C_{100}) and longer dolichols (C_{85}-C_{100}) were found in *E. guineensis* Lubuk Kertang sites: mangroves and paddy fields (Figure 2, Table 3). This polyisoprenoid profile was close to *dura* type of *E. guineensis* (Arifiyanto et al. 2017). The *dura* type has a thick- shell female fruit, and contains less CPO (Basyuni et al. 2017a). By contrast, different patterns are shown in Figure 3 and Table 3, between two families of polyprenols (shorter and longer polyprenols) and longer dolichols belonging to *pisifera* or *tenera* type of oil palm. The present findings were commensurate with our previous results that polyisoprenoids could differentiate between fruit types of oil palm (Arifiyanto et al. 2017). Furthermore, *dura* type is discernible from other types from fresh fruit bunch and their physical and chemical characteristics (Basyuni et al. 2017a).

To extend our knowledge on chemotaxonomic importance of polyisoprenoids in *E. guineensis*, similar experiments were carried out with samples from oil palm Company (PT Socfin Indonesia, PT Harapan Sawita) and smallholders around North Sumatra. Tables 4-5 summarize the results of quantitative analysis and carbon-chain length of polyprenols and dolichols concentration of *E. guineensis* in oil palm companies and randomly selected smallholders. The quantity of polyisoprenoid was largest in Socfindo *dura* and nursery leaves. The highest content of polyprenols belong to the leaves of Socfindo *tenera*. In contrast to this observation, Socfindo nursery had the highest content of dolichol (Table 4). This present pattern of polysisoprenoid in *E. guineensis* was supported by a previous study (Arifiyanto et al. 2017) showing the slightly more abundant polyprenols over dolichols (around 60: 40 in ratio).

Similar pattern of *tenera* or *pisifera* type was also observed in oil palm leaves from oil palm companies and smallholders as depicted in Figures 5-6 and Table 5. Table 5 summarizes carbon length distribution found in the leaves, divided into two: most locations had more polyprenols (shorter and longer polyprenols) and only one location (SN) had slightly more dolichols than polyprenols. However, two locations no longer had polyprenols (SN and OPA). These locations are identical to *dura* type as previously described in (Arifiyanto et al. 2017).

It has been proposed that the circumstance of accumulation of polyprenols in leaves is correlated with natural and time-dependent manner of physiological changes (Swiezewska et al. 1994). The increased accumulation of polyprenols has been shown in the old leaves of gymnosperm ginkgo (*Ginkgo biloba*) and angiosperm rubber plants (*Hevea brasiliensis*) (Tateyama et al. 1999) and senesced mangrove leaves of *Bruguiera gymnorrhiza* and *Kandelia obovata* (Basyuni et al. 2016). Furthermore, it has been reported that the pattern of polyprenyl esters was more multifarious in *Lumnitzera racemosa* old leaves (Skocylas et al. 1994). The increased abundance of polyprenols with age has been described *in vitro* plant tissue culture of *Taxus baccata* suspension cells (Skorupinska-Tudek et al. 2007). These studies indicated that leaves aging altered the profile and characteristics of plant polyprenols. These studies suggested that the biosynthetic pathways of polyprenols and dolichols are differently modulated in plant kingdom including *E. guineensis*.

Table 2. Profil of polyisoprenoid of oil palm leaves in Lubuk Kertang and campus of USU, North Sumatra, Indonesia

Location	Tissue	TL (mg/g dw)	PL (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% in total lipid			% in polyisoprenoid		Type
						Pl	Pol	Dol	Pol	Dol	
MS 0.5%	Leaves	69.6±8.8	2.7±0.9	1.5±0.6	1.2±0.4	0.4±0.0	0.2±0.0	0.2±0.0	54.4±6.3	45.6±4.4	II
MS 1%	Leaves	62.0±7.8	7.8±1.6	4.3±0.9	3.5±1.0	2.1±0.2	1.2±0.2	0.9±0.0	54.9±2.1	45.1±4.3	II
MS 2%	Leaves	65.3±7.1	7.3±2.1	3.7±1.0	3.6±1.7	1.0±0.3	0.5±0.2	0.5±0.2	50.7±2.3	49.3±3.1	II
PF 1	Leaves	63.7±4.2	8.7±1.1	3.8±0.7	4.9±1.7	0.9±0.6	0.4±0.1	0.5±0.2	43.3±1.1	56.7±2.1	II
PF 2	Leaves	63.8±4.5	5.8±3.6	2.8±1.0	3.0±2.7	2.3±0.4	0.8±0.1	1.5±0.3	49.2±0.1	50.8±3.2	II
PF 3	Leaves	64.1±9.5	9.4±0.0	5.2±0.5	4.2±0.5	1.6±0.2	0.8±0.4	0.8±0.2	54.1±4.3	45.9±2.2	II
PF 4	Leaves	54.1±2.6	9.1±2.9	4.9±0.9	4.2±2.2	1.4±0.2	0.5±0.0	0.8±0.3	54.1±4.1	45.9±1.2	II
Gate 1	Leaves	61.5±1.0	1.8±0.3	1.2±0.2	0.5±0.0	3.3±0.1	2.1±0.0	1.2±1.2	69.9±3.2	30.1±2.2	II
Gate 3	Leaves	69.7±2.6	2.7±1.4	1.7±0.7	1.0±0.6	2.3±0.3	1.4±0.1	0.9±0.0	62.4±2.1	37.6±4.4	II
Gate 4	Leaves	62.5±1.0	2.8±0.4	2.0±0.8	0.8±0.3	2.8±0.1	1.8±0.0	1.0±0.0	71.4±2.3	28.6±1.1	II
CeE	Leaves	67.2±8.7	3.2±1.2	2.1±0.5	1.1±0.7	1.4±0.2	0.8±0.0	0.3±0.0	65.4±2.3	34.6±1.1	II
CvE	Leaves	57.5±3.4	4.4±1.6	3.0±1.0	1.3±0.5	0.5±0.1	0.3±0.0	0.2±0.0	69.8±2.2	30.2±1.2	II
UL	Leaves	69.7±2.6	2.0±1.8	1.1±0.9	0.9±1.0	0.8±0.2	0.5±0.0	0.3±0.1	53.2±3.2	46.8±2.1	II
FA	Leaves	60.7±6.2	2.7±1.5	1.7±0.7	1.0±0.7	2.5±0.2	1.7±0.0	0.8±0.0	62.1±1.1	37.9±1.6	II
FD	Leaves	71.3±4.6	3.6±1.9	2.3±1.4	1.2±0.4	2.5±0.3	1.6±0.5	0.9±0.0	64.6±4.1	35.4±0.1	II
FE	Leaves	60.1±4.9	2.6±1.0	1.7±0.8	0.9±0.3	0.5±0.2	0.3±0.1	0.2±0.1	65.8±0.1	34.2±1.3	II
FM	Leaves	78.7±3.6	2.3±0.6	1.3±0.2	1.0±0.4	3.1±0.0	1.6±0.0	1.5±0.4	56.4±0.2	43.6±0.0	II

Note: The location abbreviations were defined in the note of Table 1. Data are expressed as the means ± standard error (n = 3)

Table 3. Carbon-chain lengths of polyprenol and dolichol of oil palm leaves from North Sumatra, Indonesia

Location	Tissue	Polyprenol	Dolichol
MS 0.5%	Leaves	50 55 60	85 90 95 100
MS 1%	Leaves	50 55 60	85 90 95
MS 2%	Leaves	50 55 60	85 90 95 100
PF 1	Leaves	50 55 60 85 90 95 100	80 85 90 95
PF 2	Leaves	50 55 60	85 90 95 100
PF 3	Leaves	50 55 60	85 90 95 100
PF 4	Leaves	50 55 60	85 90 95 100
Gate 1	Leaves	50 55 60 85 90 95 100	85 90 95 100
Gate 3	Leaves	50 55 60 80 85 90 95	80 85 90 95
Gate 4	Leaves	50 55 60 65 80 85 90 95	80 85 90 95 100
CeE	Leaves	50 55 60 85 90 95 100	80 85 90 95 100
CvE	Leaves	50 55 60 85 90 95 100	85 90 95 100
UL	Leaves	50 55 60 85 90 95 100	85 90 95 100
FA	Leaves	50 55 60	80 85 90 95 100
FD	Leaves	55 60 65 85 90 95 100	85 90 95 100
FE	Leaves	50 55 60 85 90 95 100	85 90 95 100
FM	Leaves	55 60 65 85 90 95 100	85 90 95 100

Note: The location abbreviations were defined in the note of Table 1

Table 4. Profil of polyisoprenoid of oil palms leaves from Socfindo and randomly selected smallholders in North Sumatra, Indonesia

Species	Tissue	TL (mg/g dw)	Pl (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% in total lipid			% in polyisoprenoid		Type
						Pl	Pol	Dol	Pol	Dol	
SD	leaves	59.3±1.0	10.3±1.7	5.6±2.2	4.7±1.5	1.7±0.1	0.9±0.1	0.8±0.2	53.9±2.1	46.1±0.2	II
SP	leaves	60.2±4.7	7.7±0.6	5.2±0.4	2.5±0.1	1.3±0.1	0.7±0.0	0.6±0.0	67.7±1.3	32.3±2.1	II
ST	leaves	59.2±2.6	10.0±0.1	5.7±0.2	4.3±0.4	1.8±0.0	1.0±0.1	0.8±0.0	56.8±1.0	43.2±1.7	II
UOP	leaves	60.4±2.8	2.4±0.1	1.7±0.2	0.7±0.1	1.9±1.8	1.1±0.8	0.8±0.5	67.1±2.8	32.9±3.2	II
SWT	leaves	60.9±3.6	9.3±0.1	4.7±2.0	4.6±1.9	1.5±0.0	0.8±0.0	0.7±0.1	50.4±1.6	49.6±1.3	II
SN	leaves	66.2±1.2	10.3±2.9	3.8±1.7	6.5±1.6	2.5±1.2	1.2±0.9	1.3±0.8	36.8±0.9	63.2±0.6	II
OPT	leaves	74.2±0.8	2.7±0.4	1.8±0.1	0.9±0.2	2.3±1.1	1.2±0.3	1.1±1.1	67.5±2.6	32.5±1.0	II
OPA	leaves	38.2±0.9	2.9±0.1	1.8±0.0	1.1±0.1	2.1±1.9	1.2±0.0	0.9±0.0	60.7±13	39.3±2.1	II
OPTHS	leaves	58.1±1.0	2.2±1.9	1.2±1.0	1.0±1.0	1.2±0.6	0.6±0.0	0.6±0.0	50.9±2.9	49.1±0.8	II
OPM	leaves	64.9±1.4	2.3±1.5	1.6±1.0	0.7±0.5	2.0±1..4	1.2±0.9	0.8±0.0	67.9±1.2	32.1±0.9	II

Note: The location abbreviations were defined in the note of Table 1. Data are expressed as the means ± standard error (n = 3)

Phylogenetic analysis of polyisoprenoids in oil palm leaves

The results of phylogenetic analysis based on carbon-chain length data of polyisoprenoids in the leaves were drawn to show the similarities land-use of oil palm. Figure 7 depicts the location similarities based on leaf polyisoprenoid carbon-chain lengths from 27 sites. These data were largely separated into two groups. The first one was a cluster of 18 sites, consisting of samples from USU (Gate 1, Gate 3, Gate 4, FE, FM, UL, CeE, CvE, and FD), oil palm company (SD, SP, ST, SD, UOP), smallholders (OPM, OPTHS, and OPT), Lubuk Kertang sites (PF4). This group represented *pisifera* or *tenera* type of oil palm. Figure 7 shows that *pisifera* and *tenera* are located close to

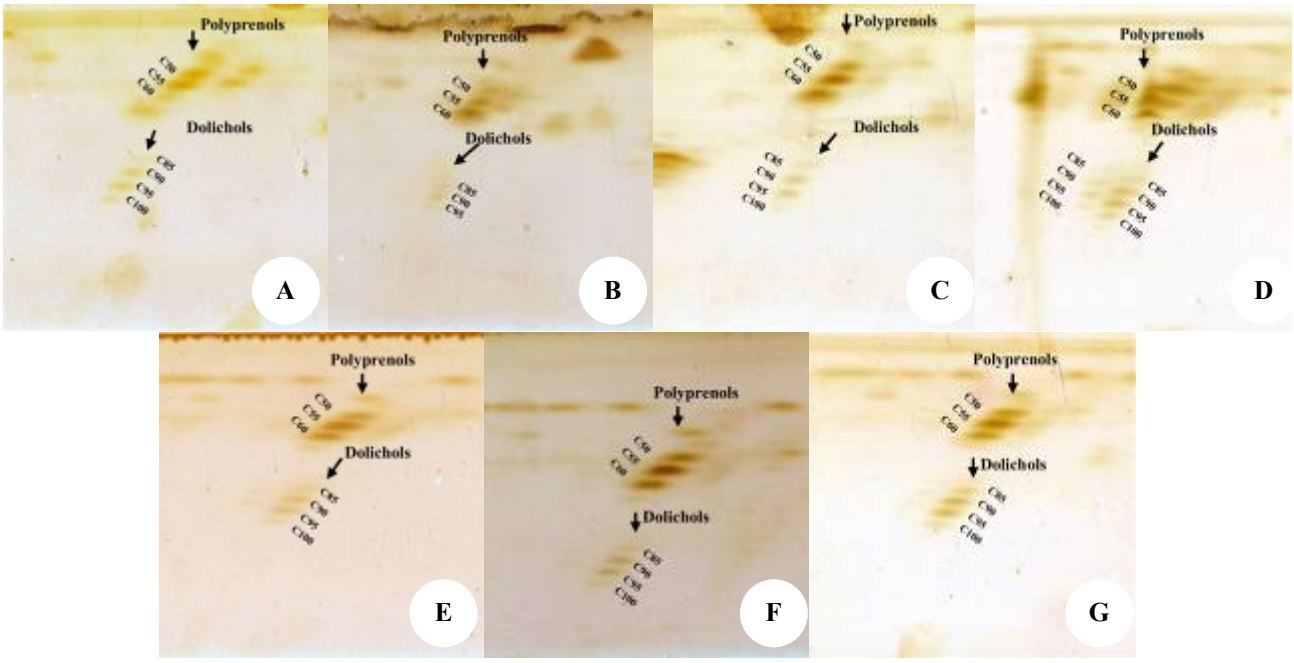

Figure 2. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from Lubuk Kertang site: MS 0.5% (A), MS 1% (B), MS 2% (C), PF 1 (D), PF 2 (E), PF 3 (F), and PF 4 (G). The location abbreviations were defined in the note of Table 1.

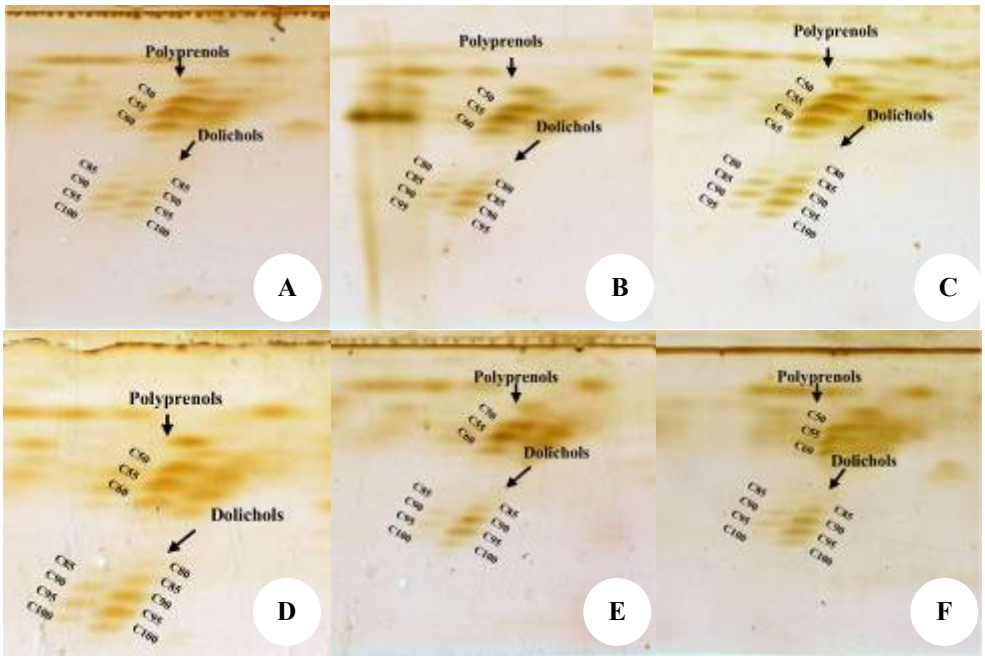

Figure 3. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from USU: Gate 1 (A), Gate 3 (B), Gate 4 (C), CeE (D), CvE (E), and UL (F)

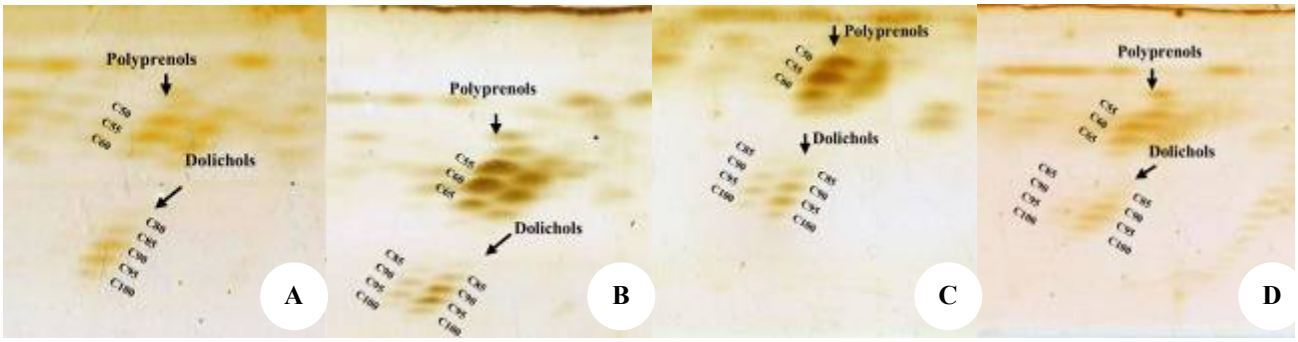

Figure 4. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from USU: FA (A), FD (B), FE (C), and FM (D)

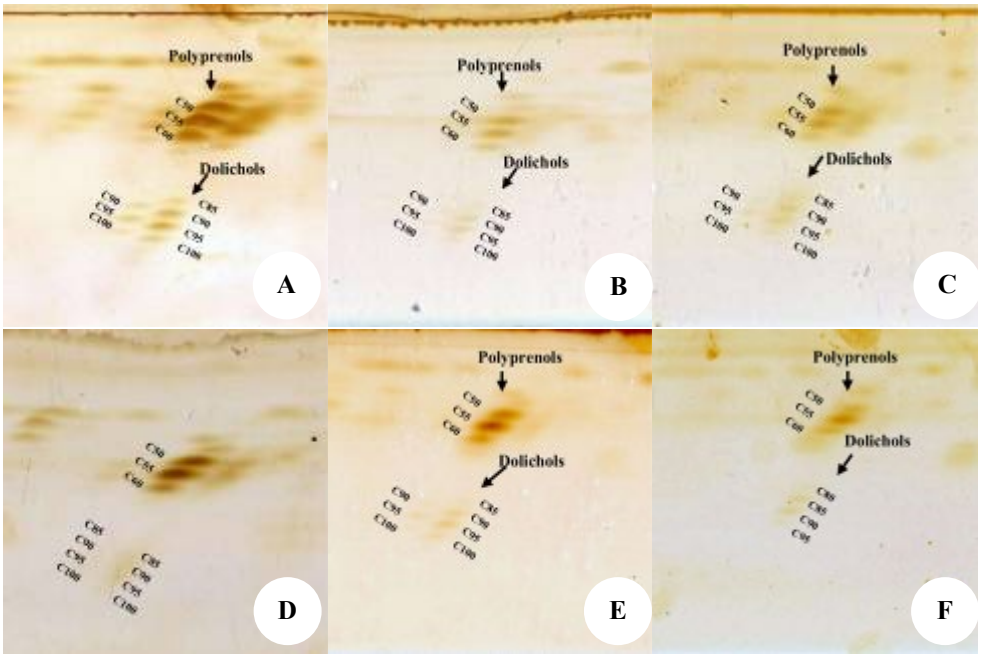

Figure 5. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from Socfindo: SD (A) SP (B), ST (C), UOP (D), SWT (E), and SN (F)

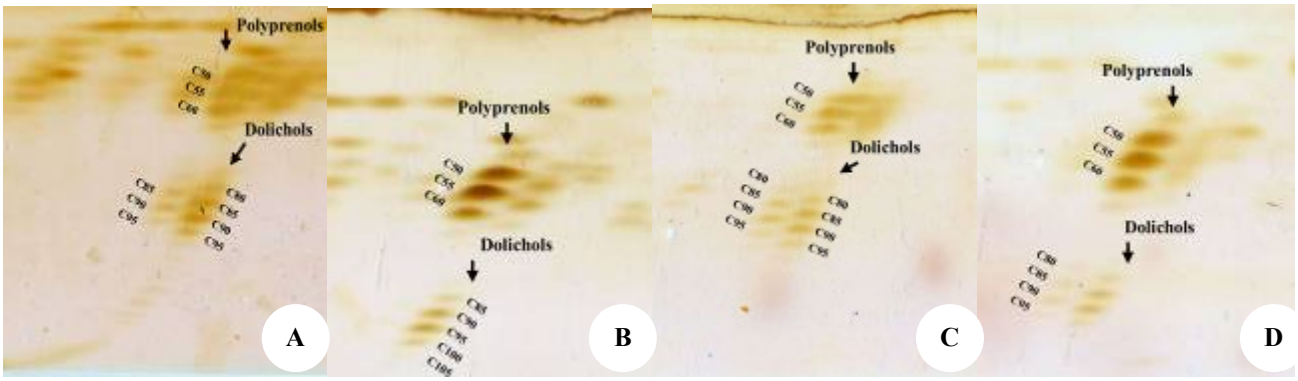

Figure 6. 2D-TLC chromatograms of hexane extracts of polyisoprenoids from small holders: OPT (A), OPA (B), OPTHS (C), and OPM (D)

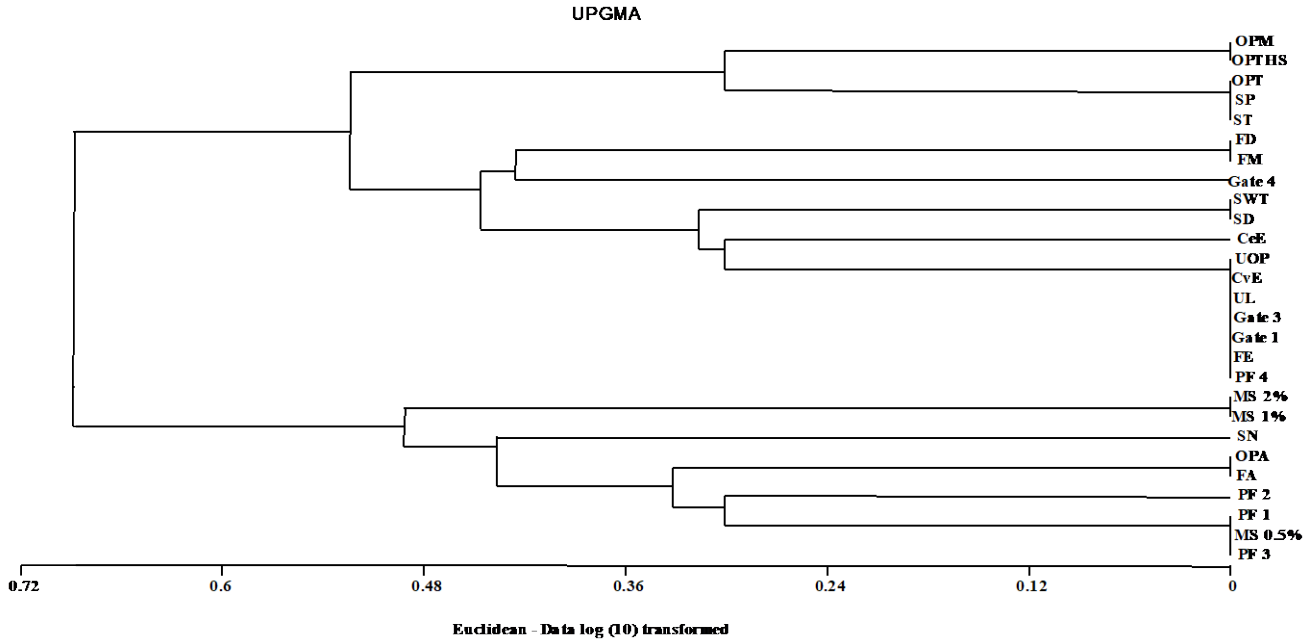

Figure 7. Phylogenetic tree showing the similarities sites of land-uses of *E. guineensis* based on carbon-chain length of leaves data of polyisoprenoids by log (10) transformation using Euclidean distance of 27 locations. The location abbreviation was defined in the note of Table 1

Table 5. Carbon-chain lengths of polyprenol and dolichol from Socfindo and randomly selected Smallholders

Location	Tissue	Polyprenol	Dolichol
SD	Leaves	50 55 60 90 95 100	85 90 95 100
SP	Leaves	50 55 60 85 90 95	80 85 90 95
ST	Leaves	50 55 60 85 90 95	80 85 90 95
UOP	Leaves	55 60 65 85 90 95 100	85 90 95 100
SWT	Leaves	50 55 60 90 95 100	85 90 95 100
SN	Leaves	50 55 60	80 85 90 95
OPT	Leaves	50 55 60 85 90 95	80 85 90 95
OPA	Leaves	50 55 60	85 90 95 100 105
OPTHS	Leaves	50 55 60 80 85 90 95	80 85 90 95
OPM	Leaves	50 55 60 80 85 90 95	80 85 90 95

Note: The location abbreviations were defined in the note of Table 1

each other and forms one cluster. Both fruits type has several analogous carbon-chain compositions. It has been shown that the differences in carbon chain-length between *pisifera* and *tenera* were from polyprenols C_{65} and C_{105}, respectively (Arifiyanto et al. 2017). It is interesting to note that oil palm company and smallholders have planted commercial type of oil palm, *tenara,* as indicated by the carbon chain-length showing the characteristics of *tenera*.

The other group was a cluster of 9 locations comprising samples from USU (FA), oil palm company (SN), smallholder (OPA), and majority Lubuk Kertang sites (MS1%, MS2%, MS3%, PF1, PF2, and PF3). The second cluster was part of *dura* type of *E. guineensis.* This finding confirmed that oil palm plantations in mangrove area and paddy field were derived from non-commercial type of oil palm plantation.

The dendrogram analysis indicated that twenty-seven sites of *E. guineensis* were grouped into appropriate types of *dura, pisifera,* and *tenera* accordingly (Figure 7), showing that the existence of polyisoprenoids in *E. guineensis* was a chemotaxonomic marker. Polyisoprenoid composition analysis could be done as an alternative method to categorize the fruit types of oil palm, besides using molecular markers (Ritter et al. 2016).

Implication to yield gap causes from planting materials

The *dura* type found in several sites may cause important yield gap in oil palm from planting materials. Yield gap has been studied to explore the possibilities for improving land productivity. It has been reported that contaminations of *dura* in the oil palm plantation reduced 35-50% bunch oil content (Woitties et al. 2017). Woitties et al. (2015) have reported oil palm in Sintang, West Kalimantan, Indonesia, that more than 50% oil palm plantation were contaminated with *dura* material, suggesting that the planting material was of inferior quality. Moreover, in Ramin, Jambi, the oil palm plantation tested was contaminated with *dura* and the yield was reduced by 30-50% of the bunches (Woitties et al. 2015).

The poor planting materials of oil palm plantation in mangrove area are noteworthy, because oil palm plantation is one of proximate drivers of deforestation in mangroves. In Indonesia, oil palm contributed 15% of mangrove deforestation (Richards and Friess 2016). On the other hand, in North Sumatra province, Indonesia, oil palm plantation became the fourth proportion of deforested secondary mangrove forest converted to other land uses

during the period of 1990-2015 (Basyuni et al. 2018b). In Lubuk Kertang site, Langkat, North Sumatra, oil palm plantation was a driver of 44% of mangrove loss from 2006-2016 (Basyuni et al. 2018c). The present study, therefore, may answer the question why Indonesian palm oil productivity is lower than Malaysia. It may be due to the poor planting materials: the contamination of *dura* type, as non-hybrid seeds.

In conclusion, the pattern of polyprenols and dolichols in the leaves of oil palm (*Elaeis guineensis*) plantations in different land-uses in North Sumatra, Indonesia showed only one type polyisoprenoids, namely type-II, having the presence of both polyprenols and dolichols. The occurrence of ficaprenols (C50-C60) and longer polyprenols (C85-C100), and dolichols (C85-C100) differentiated the *E. guineensis* types (*dura, pisifera*, and *tenera*). The cluster analysis demonstrated that twenty-seven sites of *E. guineensis* were grouped into appropriate types of *dura, pisifera*, and *tenera* accordingly, indicating that the occurrence of polyisoprenoids in *E. guineensis* was a chemotaxonomic criterion. The finding of polyisoprenoid profile of *E. guineensis* as *dura* type (non-commercial) in mangrove area and paddy field sites may reveal significant causes of yield gap in oil palm plantation from planting materials.

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