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Polyprenol and Dolichol Content in the Seed Tissues of *Elaeis guineensis* Jacq. from Commercial Seeds

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ABSTRACT

Polyisoprenoid alcohols are found in the cells of all living organisms. This work aims to investigate their distribution in the seed tissues of oil palms in commercial and non-commercial companies using two-dimensional thin layer chromatography (2D-TLC). The distribution of dolichols and polyprenols found in oil palm plants varies depending on the region. There is no difference in the distribution of polysoprenoids between Socfin Indonesia (Socfindo) CI Ganoderma susceptible, Socfindo Lame, and Indonesian Oil Palm Research Institute (IOPRI) BSY2C seeds tissue (4.5 mg/g dry weight). The total polyprenol and dolichol in the seed tissues of oil palms from Socfindo MTG was higher than those from other oil palms: it was 6.1 mg/g dry weight and 4.7 mg/g dry weight, respectively. The highest polyisoprenoids concentration in the seed tissues of oil palms was found in those from Socfindo Yagambi. Polyprenol; longer chain lengths were observed in the oil palms of non-commercial (C75-C100) and dolichol of commercial seeds from IOPRI 05-73 (C70-C100). The seeds from Socfindo Yagambi were clearly separated from other samples, however, the seeds from commercial and non-commercial seeds (IOPRI B SMB and oil palm PT Harapan Sawita (OPHS) respectively were observed in one group. This study indicated that there are distinct groups of polyisoprenoid carbon chains randomly derived from cluster analysis in the origin of oil palm seeds.

Keywords: Polyisoprenoid, polyprenol, dolichol, commercial seeds, non-commercial seed.

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INTRODUCTION

Polyisoprenoid alcohols are found in the cells of all living organisms including bacteria, human, and plants. Polyprenols are mainly present in plant photosynthetic tissues, whereas dolichols are present in plant roots [1][2]. Bioactive long-chain isoprenoid alcohols (polyprenol) occur in plants. Polyisoprenoid (polyprenol and dolichol) has been potent to anticancer and antibacterial activities [3]. Depending on the source plants, the chain length of natural polyprenols varies from six to forty isoprene units [4][5]. Polyprenols may play the role of scavengers for reactive oxygen and nitrogen species, protecting cells to oxidative stress. Un-saturated plant polyprenols and dolichols have the foremost antioxidant potential [6]. Polyprenols have been reported as important active lipid components such as antivirus, hepatoprotective, and antitumor in pharmacology [7].

Natural compounds classified as products of secondary metabolism are widely studied for their possible cellular functions of polyisoprenoids [8]. The seeds from monocotyledonous plants contained dolichol, a homologous varied of alpha-saturated polyisoprenoids alcohols, polyprenol, and a homologous series of alpha-unsaturated polyisoprenoid alcohols [9]. Dolichol synthesis established for the means asparagine-linked glycoproteins, such as tunicamycin in the establishment of GlcNAc-P-P-dolichol catalyzed by the enzyme dolichol phosphate N-acetylglucosamine-1-phosphate transferase [10][11]. The long-chain polyisoprenoid alcohols (polyprenols and dolichols), a cluster of compounds reported to develop in plants, indicates a major heterogeneity in terms of carbon-chain length [12].

Oil palm plantation (*Elaeis guineensis*) is the main commodity that supports the Indonesian economy and is well known as a profitable business [13][14]. According to our previous studies on the polyprenols of C45-C65 and C90-C105 length chain, dolichols with C85-C105 chain-length have been reported in oil palm leaf tissues as a chemotaxonomic marker [15]. Recently, we reported the carbon chain pattern in *E. guineensis* polysioprenoids leaves from different land uses, showing the yield gap in oil palm from planting materials [16]. The present study, therefore, extends our work to investigate the polysoprenoids in oil palm seeds from the commercial and non-commercial companies to obtain more insights distribution and occurrence of polyprenol and dolichol from oil palm.

MATERIALS AND METHODS

Plant Materials

Seventeen oil palm seed sources with triplicate were sampled in North Sumatra, Indonesia. These were collected as non-commercial seeds from Universitas Sumatera Utara (USU), a mangrove area 1% of salinity (MS1%), oil palm from smallholder in Marihat, Serdang Bedagai, North Sumatra (OPM), and private company of PT Harapan Sawita, Langkat, North Sumatra (OPTHS), whereas the oil palm seeds from Socfindo, Serdang Bedagai, North Sumatra (private company), and Indonesian Oil Palm Research Institute (IOPRI), Medan, were categorized as commercial seeds as shown in Figure 1. The seeds were kept in an average temperature at 25–27°C and the humidity was 83-84%.

Chemical Compounds

A mixture of dolichol (C95-C110) standard compound with a combination of polyprenol (C90-C100) was used to characterize the polyisoprenoids [1][17]. The silica gel 60 TLC plates and reversed-phase of silica RP-18 HPTLC plates were obtained from Merck.

Polyisoprenoid Alcohols Isolation

The protocol for the separation of polyisoprenoids was done as reported earlier [1][14]. The seeds of oil palm species were kept in an oven at 60 °C for two days. The dried tissue (1–2 g) was pressed to powder and kept afloat in a 2/1 (v/v) chloroform/methanol solution for two days. The extract of lipid was saponified at 65°C for 24 h in 50% ethanol with 2 M KOH. The un-saponified lipids from a tissue sample were extracted into hexane, and the organic solution was evaporated and dissolved again in hexane. The seeds extracted (±100 µg) were used for each TLC plate.

Two-Dimensional Thin Layer Chromatography (2D-TLC) Analysis

1D-TLC was formed 45 minutes earlier in a 20 cm × 3 cm (silica gel) plate of glass in a soluble system of toluene-ethyl acetate (9:1) as was done previously [1]. The 2D-TLC reversed-phase C18 silica gel was finished with acetone as the soluble for 45 min. The state of the dilute polyisoprenoid alcohols from the 2D-TLC was tough and envisaged with iodine evaporate. To clarify the family to dolichols or polyprenols, a standard was applied to the line of sample for the 1D-TLC. The occurrence of one family was verified in 2D-TLC. The standard was used on the sample and delivered with a solution system as previous reported. The chromatographic images were obtained and digitally scanned with Canon G-2000 printer series. The polyisoprenoid group was clarified by the correlation of movement on TLC in authentic dolichol or polyprenol standards using the 2-Dimensional phase. The polyprenols and dolichols found on the HPTLC plates RP-18 were measured with ImageJ ver. 1.46r [15] with dolichol and polyprenol standards.

Phylogenetic Analysis

Phylogenetic analysis was carried out on a selected data of 17 commercial and non-commercial seeds in palm oil from the data of carbon-chain lengths of polyisoprenoids by log (10) transformation by Euclidean distance [18]. Dendrograms indicated the relationship by clustering analysis used in the Un-Weighted Pair Group Method with Arithmetic mean (UPGMA) MVSP ver. 3.22 software multivariate statistical package (Kovach Computing Service).

RESULTS AND DISCUSSION

The lipids have a biological function in the cells of the plant as integral components of membranes. They regulate the metabolic processes or signalling molecules and are essential for plant viability [19][20]. Polyisoprenoids have a crucial role in post-translational protein modifications as the required saccharide bearer in the biosynthesis of glycosyl-phosphoinositol anchor, protein N-, O- and C-glycosylation [21][22].

Polyprenols are bioactive long-chain isoprenoid alcohols that ensue in various plants including oil palm. The minor components in the crude palm oil are carotenoids, vitamin E, squalene, sterols, triterpenic alcohols, dolichols and polyprenols, ubiquinones, phospholipids, methyl sterol, and glycolipids [14][23]. Yamaguchi *et al.* (2019) have reported the presence of natural rubber in 35 polyisoprenoid biosynthesis pathways and methods for industrial rubber products to produce a polyisoprenoid using isoprenoid from harvested *Hevea brasiliensis* [24]. Other reported methods for producing a polyisoprenoid using a gene coding for a cis-prenyl-transferase protein group involve s gene coding the Nogo-B receptor protein for elongation factor. This is a method for producing a polyisoprenoid in vitro *H. brasiliensis* [25].

In the polyprenol reductase pathway, polyprenols may work as scavengers not only for reactive oxygen and nitrogen species but also for protecting cells from oxidative stress [26]. The appearance and occurrence of polyisoprenoids are used as chemotaxonomic marker [1][27]. Based on the molecular insights of polyprenol phosphates with diverse membrane-associated proteins in glycoconjugate, a cis/trans geometry in polyprenol phosphate substrates of biosynthesis has been reported [28].

The profile of polysoprenoids in non-commercial seeds is described in Table 1. The total number of lipids in oil palm tissues was high in the USU region. It was 62.7 mg/d dry weight, whereas, in oil palms, from mangrove areas (1% of salinity), the lipid value was 30.4 mg/d dry weight. The oil palm from a smallholder in Marihat (OPM) and PT Harapan Sawita (OPTHS) was 20.5 mg/d dry weight and 18.6 mg/d dry weight respectively. There was a difference in polysoprenoids between the origins of oil palm crops; the range value was 3.3 to 5.7 mg/g dry weight. The quantity of polyprenol was highest in the oil palm from Marihat (smallholder) seeds tissue (3.5 mg/g) and the lowest in the ones from mangrove area (1% of salinity) (1.4 mg/g).

There was no difference in the dolichol content from USU and OPTHS tissue of seeds palm; the value was 1.6 mg/g, but the total dolichol content from the mangrove area was 2.0 mg/g, which is less than OPM, which is 2.2 mg/g. The polysoprenoid in total lipid ranges from 1.9% to 2.2%. The polyprenol from the oil palms within the USU area was higher than the other oil palms, which was 1.8%. Furthermore, the dolicol of oil palm within the mangrove area (1% of salinity) was higher than the other, which ranges from 0.4% to 1%. Total polyprenol and dolycol in polysoprenoids content of oil palm from USU and OPM was the same value, it was 62% and 37%, respectively. In this study, we found that the polyprenol and dolichol in the seed tissues of oil palm were on the same type within each non-commercial origin and that it was type II distribution.

Table 3 shows the polyisoprenoid distribution in the oil palms of commercial seeds. The highest total lipid content in seed tissues was found to be in Socfindo Lame species: 12.1 mg/g dry weight, followed by Socfindo CI Ganoderma susceptible and Socfindo Yagambi – 11.6 mg/g dry weight and 11.2 mg/g dry weight, respectively. The weight of total lipid from IOPRI in the seed tissues of the oil palm ranged from 8.5 to 10.7 mg/g dry weight.

On the other hand, there was no difference in polysoprenoids between Socfindo CI Ganoderma susceptible, Socfin Lame, and IOPRI BSY2C (4.5 mg/g dry weight). The total polyprenol and dolychol in the seed tissues of oil palms from Socfin MTG was higher than the other oil palms: 6.1 mg/g dry weight and 4.7 mg/g dry weight, respectively. The highest polysoprenoid concentration in the seed tissues of oil palm was found in the seed tissues from Socfin Yagambi, whereas the seeds of oil palm from Socfindo CI Ganoderma the lowest value 8.1 and 2.6 mg/g dry weight, respectively. In contrast to the concentration of polyprenol in the total lipid, the same value measure of polyprenol was found in the seed tissues from Socfin Lame and IOPRI KEL10.DYP (1.9%). The dolichol concentration in the total lipid varied ranging from 1.3% to 4.3%, except the seeds from IOPRI 05-73B and IOPRI TCT 2.4%, respectively.

The polyprenol in the concentration of polysoprenoid seed tissues from Socfindo C3 Ganoderma resistance, Socfin MTG, Socfin Yagambi, Socfin Lame, IOPRI 718-C and IOPRI 239-C were higher than dolichol. There was no detection of polyprenol concentration from IOPRI BS Y2C, but the dolichol concentration was 100%. The seed tissues of oil palm from IOPRI BS Y2C was found as type I, but the other samples were classified as type II.

Carbon-Chain Length of Polyprenol and Dolichol

The carbon-chain lengths of polyisoprenoid in the oil palm is shown in Table 2. It varied according to the origin growing place type. In the seeds of the oil palm tissue from USU, there is an occurrence of carbon-chain polyprenols C75-C100 and dolichols C80-C100. However, carbon chains of polyprenol C80-C95 and dolichol C85-C95 were observed in the seeds of MS1%. In the seeds of OPM, the carbon-chain polyprenols C80-C100 and dolichols C85-C100 were detected, but in the OPTHS, there only a short carbon-chain of polyprenols C75-C85 and dolichol C75-C90 were detected. A difference in the carbon-chain chromatograms was detected in the seeds of oil palms from four origins (Figure 2).

Regarding the carbon-chain length of polyisoprenoid from commercial seeds, a longer chain of polyprenol (C80-C105) and dolichol (C80-C110) was found in the seeds from Socfindo Yagambi (Table 4). Furthermore, a shorter chain of polyprenol (C75-C85) and dolichol (C70-C85) was found in the seeds from Socfin IOPRI B SMB. Polyprenol was not found in the chain in the seed tissues from IOPRI BS Y2C, but dolichol (C80-C95) was found. Moreover, the similarity of polyprenol and dolichol (C80-C100) was observed in seed tissues from IOPRI 718-C and IOPRI CTC.

The analysis of polyisoprenoid in the seeds of oil palm plants showed that the majority of polyisoprenoid alcohols are polyprenols and dolichols except for the seeds of IOPRI BS Y2C (commercial seeds), as shown in Table 2 and 4. Depending on the source, the chain length of natural polyprenols varied from C45 to C100 in oil palm leaves and root tissues [29]. Furthermore, according to Arifiyanto *et al* (2017), the fruit shell of palm oil contains a longer chain of dolichol (C85-C110). Moreover, longer polyprenol and dolichol were observed in the non-commercial seeds of oil palms from USU (Figure. 1).

Figures 3 and 4 show the 2D-TLC chromatograms of polyisoprenoid for oil palm tissues in commercial seeds. Figure 3.A; 4.B; 4.C; and 4.D show that a same chain length of polyprenol (C80-C100) was found. In contrast, Figure 3.C; 3.E; 4.A; 4.C and 4.E show that a same chain length of dolychol (C80-C100) was found in the seed tissues of oil palms. The cluster analysis of the polyisoprenoid of carbon chains was analysed into a dendrogram using the UPGMA method by log (10) transformation by Euclidean distance (Figure 5). The samples were clustered into two large groups of seed tissues. The seeds from Socfindo Yagambi were clearly separated from the other samples. Further groupings were observed in the same group in samples from commercial and non-commercial seeds (IOPRI B SMB and OPTHS). Meanwhile, the grouping between IOPRI 293C and Socfindo C3 Ganoderma resistant seeds tissue were closest in one cluster. This result indicated that there are distinct groups of polyisoprenoid carbon chains randomly clustered in the origin of oil palm crops. However, the other cluster showed one group between IOPRI CTC and IOPRI 718-C, but a separated group with seeds from Marihat (smallholder), as expected.

To confirm the role of polyisoprenoids in terms of long chain polyprenol and dolichol, we analysed the polyisoprenoid chromatogram of seed tissues from commercial and non-commercial oil palms belonging to type II. According to Basyuni *et al.* (2017), the distribution of polyprenols and dolichols in the plant tissue was classified into three types. In type I, the distribution of dolichol was more than polyprenol (>90%); type II showed a balanced occurrence of polyprenol and dolichol, and in type III, the polyprenol majority than dolichol (> 90%) [1]. The present study supports the previous findings on consistent carbon chain length in different tissues of oil palms (leaves, fruits mesocarp, fruits shell) and plant stage (seedling and mature) which belong to type-II, and confirms the presence of polyprenol and dolichol [15][16].

There are no different types of polyisoprenoid profiles (type-II) in the seeds, except IOPRI BS Y2C. The chromatograms of polyisoprenoid for oil palm tissue in some commercial seeds indicated the same chain length of polyprenol and dolichol. Therefore, these commercial seeds could be confirmed as quality seeds, *tenera* type, hybrid between *dura* (female) and *pisifera* (male) and is a basis for commercial oil palm in Indonesia, whereas, no difference in carbon-chain lengths was observed in non-commercial seeds. A cluster analysis of polyisoprenoid in the seed tissues of the oil palm origin plants were distinct from that of commercial and non-commercial seeds. The distribution of polyisoprenoids in oil palm leaves could be an alternative way to categorize oil palm fruits [29].

However, in this study, oil palm seeds have limited results to differentiate between commercial and non-commercial seeds. Further investigations, therefore, there are required to address this answer, using more large samples from different oil palm seeds producers in Indonesia.

CONCLUSION

The consistent polyisoprenoid pattern in oil palm seeds in the carbon chain length belong to type-II with other oil palm tissues, suggesting that the presence of polyisoprenoids is a chemotaxonomic marker. This study indicates the presence of distinct groups of polyisoprenoids randomly derived from cluster analysis in the origin of oil palm seeds with limitation to differentiate between commercial and non-commercial seeds.

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Table 1: Polyisoprenoid profile in non-commercial oil palm seeds

Species	Tissue	TL (mg/g dw)	PI (mg/g dw)	Pol (mg/g)	Dol (mg/g)	% in total lipid			% in polyisoprenoid		Type
						PI	Pol	Dol	Pol	Dol	
USU	seed	62.7±5.4	4.2±0.8	2.6±0.5	1.6±0.3	2.2±0.0	1.8±0.2	0.4±0.0	62.1±3.2	37.9±1.2	II
MS1%	seed	30.4±3.7	3.4±1.5	1.4±0.8	2.0±0.7	3.2±1.2	1.5±0.2	1.7±0.1	41.6±3.2	58.4±1.1	II
OPM	seed	20.5±0.5	5.7±2.3	3.5±1.6	2.2±0.6	3.0±0.1	1.7±0.0	1.3±0.2	62.3±1.2	37.7±1.1	II
OPTHS	seed	18.6±0.4	3.3±1.9	1.7±0.9	1.6±0.9	1.9±0.1	1.0±0.0	0.9±0.0	52.2±0.2	47.8±1.2	II

TL: total lipid, PI: polyisoprenoids, Pol: polyprenol, Dol: dolichol. USU: oil palm from University Sumatera Utara, MS1%: oil palm from mangrove area (1% of salinity), OPM: oil palm from Marihat (smallholder), OPTHS: oil palm from PT Harapan Sawita.

Tabel 2: Carbon-chain lengths of polyprenol and dolichol non-commercial oil palm seeds

Species	Tissue	Polyprenol	Dolichol
USU	seed	75 80 85 90 95 100	80 85 90 95 100
MS1%	seed	80 85 90 95	85 90 95
OPM	seed	80 85 90 95 100	85 90 95 100
OPTHS	seed	75 80 85	75 80 85 90

USU: oil palm from University Sumatera Utara, MS1%: oil palm from mangrove area (1% of salinity), OPM: oil palm from Marihat (smallholder), OPTHS: oil palm from PT Harapan Sawita.

Tabel 3: Profile of polyisoprenoid in seeds from Socfindo and IOPRI

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	
Socfindo CI Ganoderma susceptible	seed	11.2±0.5	4.5±0.6	2.1±0.2	2.4±0.3	2.6±0.0	1.2±0.1	1.4±0.0	46.4±3.2	53.6±2.1	II
Socfindo C3 Ganoderma resistance	seed	11.7±1.4	5.6±4.0	2.9±0.7	2.7±3.2	5.0±1.2	2.7±1.2	2.3±0.1	52.9±2.8	47.1±1.1	II
Socfin MTG	seed	13.6±4.0	10.8±8.8	6.1±5.5	4.7±3.3	7.3±0.4	4.1±0.2	3.2±0.4	56.6±0.1	43.4±2.1	II
Socfin Yagambi	seed	11.6±0.3	9.6±10.	5.3±3.8	4.3±4.8	8.1±0.8	4.5±0.4	3.6±0.4	55.0±2.1	45.0±1.6	II
Socfin Lame	seed	12.1±0.5	4.5±0.6	2.3±0.2	2.2±0.4	3.7±1.1	1.9±0.0	1.8±0.0	50.8±3.1	49.2±2.9	II
IOPRI KEL 10. DYP	seed	9.8±0.5	4.6±0.5	1.9±0.1	2.7±0.3	4.7±0.0	1.9±0.0	2.8±0.0	41.3±3.2	58.7±2.6	II

IOPRI 05-73 B	seed	9.5±2.1	4.4±0.0	2.1±0.4	2.3±0.3	4.8±0.1	2.3±0.0	2.4±0.0	48.1±2.0	51.9±3.1	II
IOPRI 718-C	seed	10.7±2.5	3.0±0.7	1.6±0.2	1.4±0.2	3.0±0.1	1.6±0.1	1.4±0.0	53.3±1.1	46,7±0.1	II
IOPRI CTC	seed	8.3±1.3	2.8±0.5	0.9±0.5	1.9±1.1	3.5±0.1	1.0±0.0	2.4±0.1	32.5±2.8	67.5±2.1	II
IOPRI BS Y2C	seed	10.2±0.9	4.5±0.7	nd	4.5±0.7	4.3±0.0	nd	4.3±0.0	nd	100±0.0	I
IOPRI 239C	seed	9.5±0.6	2.9±0.0	1.7±0.5	1.2±0.4	3.1±0.0	1.8±0.0	1.3±0.0	58.8±2.1	41.2±1.1	II
IOPRI B SMB	seed	10.4±0.4	3.7±0.5	1.7±0.1	2.0±0.4	3.5±0.0	1.6±0.0	1.9±0.0	45.5±1.0	54.5±1.9	II
IOPRI 1-54 D	seed	10.0±0.9	4.0±0.6	2.0±0.1	2.0±0.4	4.1±0.1	2.0±0.0	2.1±0.0	49.6±2.9	50,4±2.0	II

Tabel 4: Carbon-chain lengths of polyprenol and dolichol in seeds from Socfin and IOPRI

Species	Tissue	Polyprenol	Dolichol
Socfindo CI Ganoderma susceptible	Seed	80 85 90 95 100	75 80 85 90 95 100
Socfindo C3 Ganoderma resistance	Seed	75 80 85 90 95	75 80 85 90 95 100 105
Socfindo MTG	Seed	80 85 90	80 85 90 95 100
Socfindo yangambi	Seed	80 85 90 95 100 105	80 85 90 95 100 105 110
Socfindo Lame	Seed	80 85 90 95	80 85 90 95 100
IOPRI KEL 10. DYP	Seed	75 80 85 90 95	80 85 90 95 100
IOPRI 05-73 B	Seed	80 85 90 95 100	70 75 80 85 90 95 100
IOPRI 718-C	Seed	80 85 90 95 100	80 85 90 95 100
IOPRI CTC	Seed	80 85 90 95 100	80 85 90 95 100
IOPRI BS Y2C	Seed		80 85 90 95
IOPRI 239C	Seed	75 80 85 90 95	75 80 85 90 95 100
IOPRI B SMB	Seed	75 80 85	70 75 80 85
IOPRI 1-54 D	Seed	75 80 85 90	75 80 85 90 95

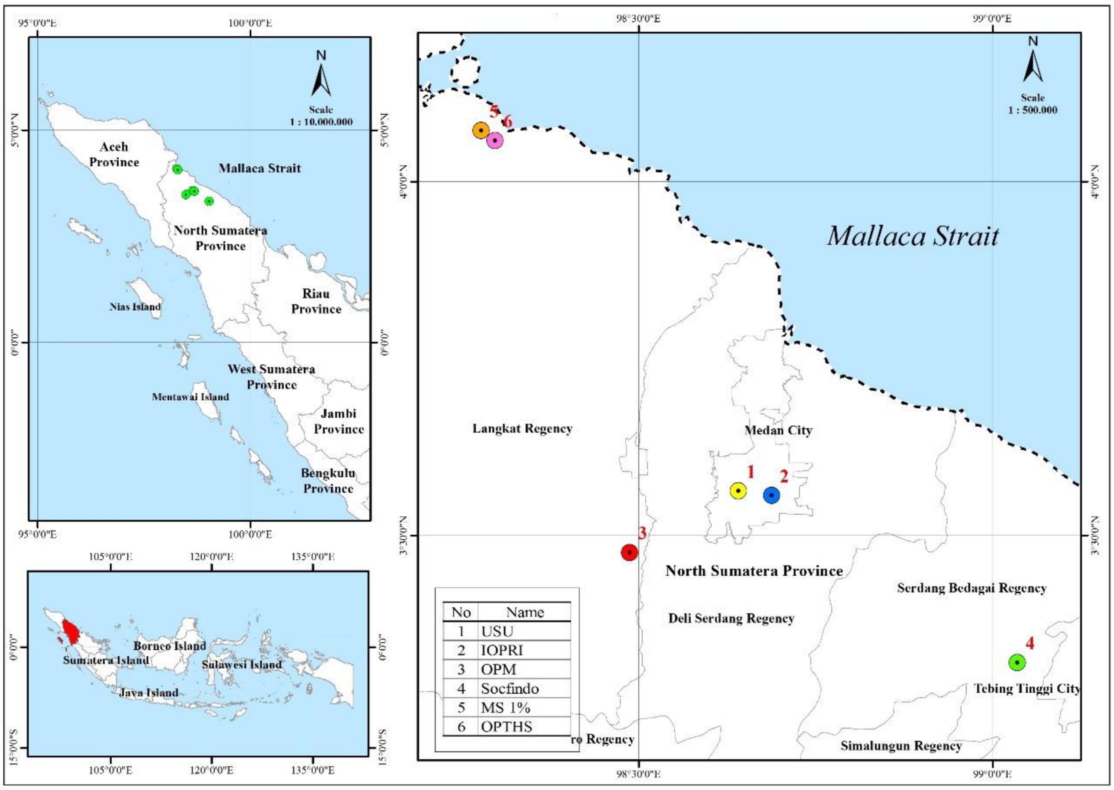

Fig 1: Map showing the study area, the seeds sampling locations, and the detailed description of the sampling sites in North Sumatera, Indonesia. USU: Universitas Sumatera Utara, IOPRI: Indonesian Oil Palm Research Institute, OPM: oil palm from small holder in Marihat, North Sumatra, Socfindo: Socfin Indonesia, MS 1%: oil palm from mangrove with salinity 1%, OPTHS: oil palm from PT Harapan Sawita, Langkat, North Sumatra, Indonesia

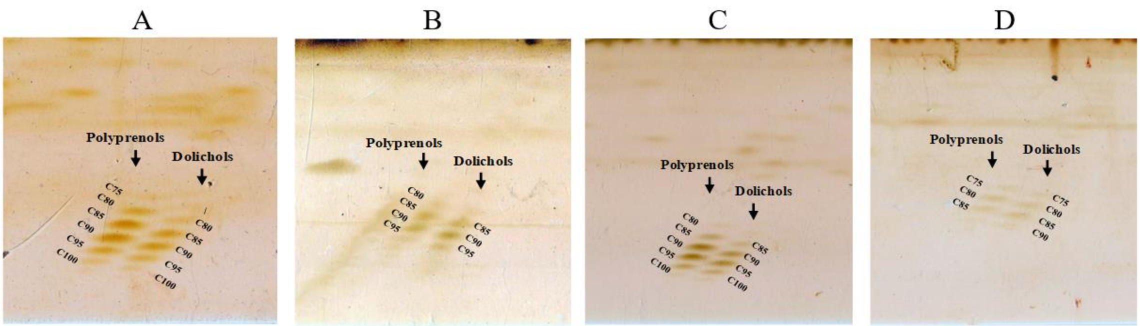

Fig 2: 2D-TLC chromatograms hexane extracts of polyisoprenoids (A) Oil palms from University Sumatera Utara (USU), (B) Oil palms from mangrove area with 1% of salinity (MS1%), (C) Oil palm from Marihat (OPM), (D) Oil palm from OPTHS.

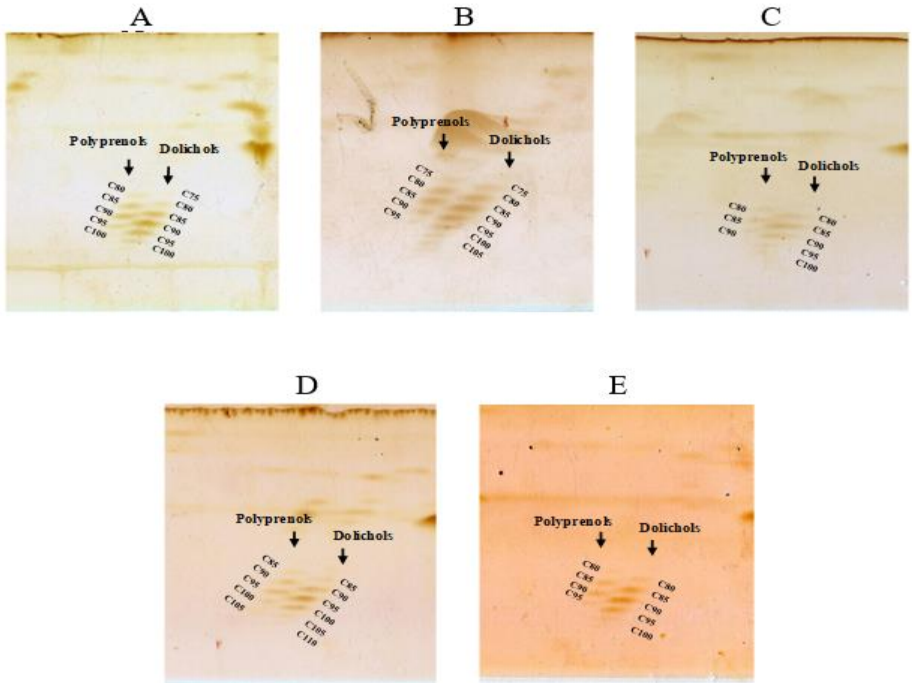

Fig 3: 2D-TLC chromatograms hexane extracts of polyisoprenoids (A) Socfindo CI Ganoderma susceptible, (B) Socfin C3 Ganoderma resistance, (C) Socfin MTG, (D) Socfin Yagambi, (E) Socfin Lame.

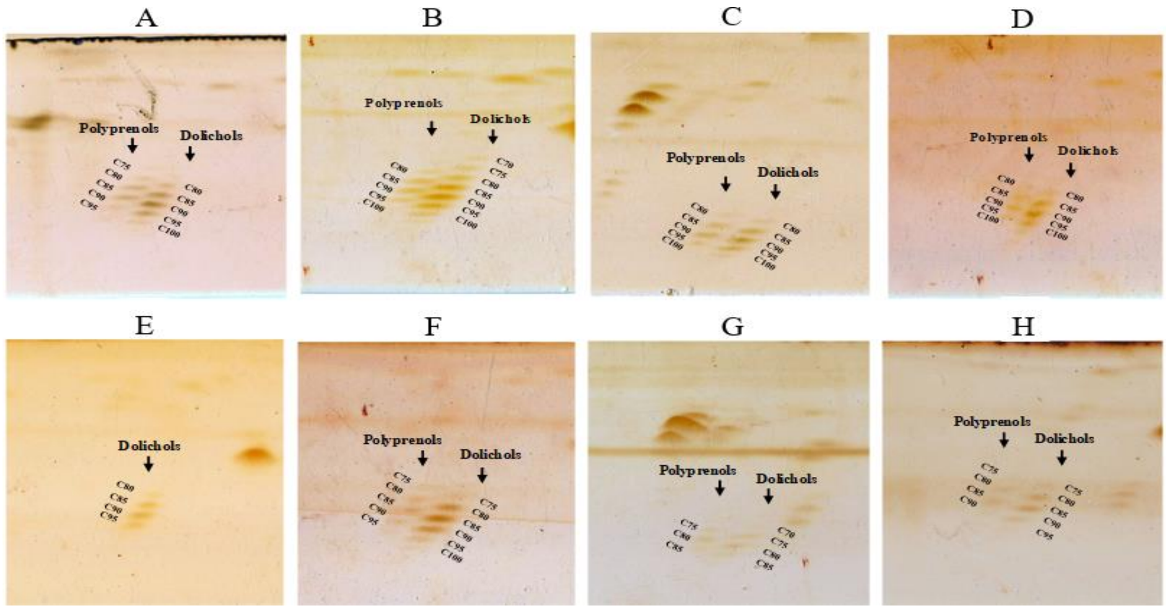

Fig 4: 2D-TLC chromatograms hexane extracts of polyisoprenoids (A) IOPRI KEL 10. DYP, (B) IOPRI 05-73 B, (C) IOPRI 718-C, (D) IOPRI CTC (E) IOPRI BS Y2C (F) IOPRI 239C (G) IOPRI B SMB, (H) IOPRI 1-54 D.

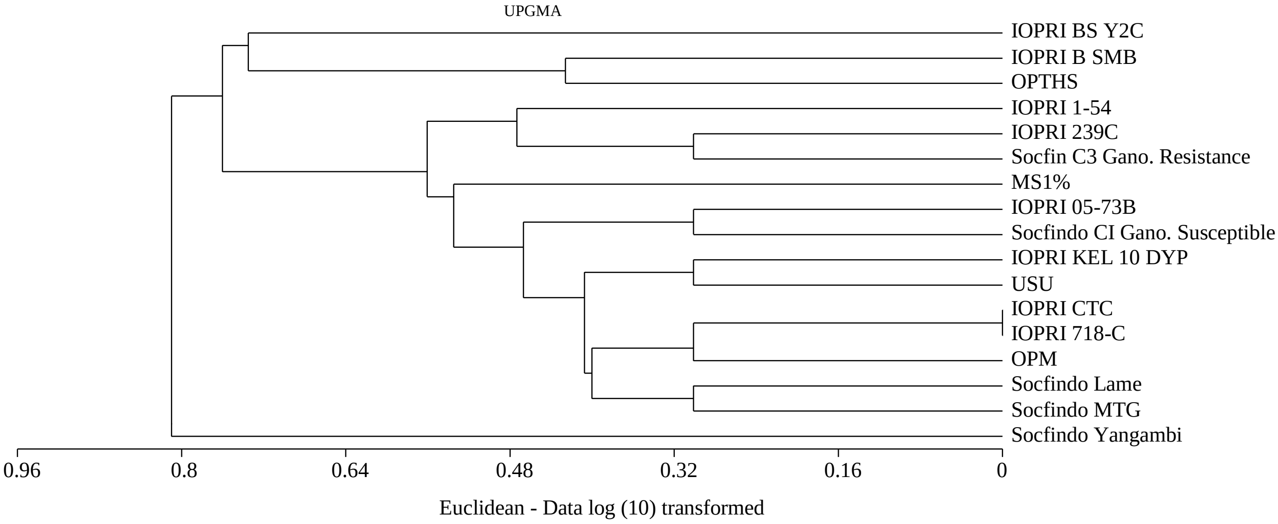

Fig 5: Dendrogram showing the relationship among 17 commercial and non-commercial seeds in palm oil from the data of carbon-chain lengths of polyisoprenoids by log (10) transformation by Euclidean distance. USU: oil palm from Universitas Sumatera Utara, MS1%: oil palm from mangrove area (1% of salinity), OPM: oil palm from Marihat (smallholder), OPTHS: oil palm from PT Harapan Sawita.