

Distribution of polyprenol and dolichol in oil palms from *Pisifera* parents and mature plants from tissue culture propagation

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Abstract. Kartika EBA, Siregar LAM, Basyuni M. 2021. Distribution of polyprenol and dolichol in oil palms from *Pisifera* parents and mature plants from tissue culture propagation. *Biodiversitas* 22: 3423-3436. Oil palm tissue culture is carried out through indirect embryogenesis, which causes somaclonal diversity to occur at the in vitro propagation stage, especially in the callus growth phase. In the cells of all living organisms can be found a group of polyisoprenoid compounds. This study aims to determine variations in oil palm plants resulting from tissue culture based on the presence of polyisoprenoid compounds. Oil palm leaf samples (*Elaeis guineensis* Jacq.) were collected from the plantation of PT. Socfin Indonesia, North Sumatra, Indonesia. Sample extraction, saponification, isolation of polyisoprenoid compounds, and two-dimensional thin-layer chromatography analysis were carried out to obtain data related to total lipids, polyprenol and dolichol profile, and Carbon (C) chain length polyprenol and dolichol from oil palm leaves of *Pisifera* parents and their propagated derivatives by indirect embryogenesis. The results showed that the amount of polyisoprenoid mother S24 was 2.41 mg/g higher than that of the parent S8 at 2.17 mg/g dry weight. Total polyisoprenoid from S8 plants in vitro ranged from 0.71 - 8.53 mg/g dry weight, with the lowest total polyisoprenoid found at S894/22 and the highest was found at S893/2. While for total polyisoprenoid from plant tissue culture of *Pisifera* S24 ranged from 0.73 - 8.05 mg/g dry weight, with the lowest total polyisoprenoid found at S24H14 and the highest was found at S24H16. The parent plants of *Pisifera* S8 and S24, as well as plants resulting from tissue culture, were categorized as having lipid pattern type II, which showed a balanced distribution of polyprenol with dolichol. The longest carbon chain was found in vitro plants S8 93/4 ranged from C50-C110, while the shortest was found in plants produced in vitro S24H7 starting from C45-C55. There were variations in the carbon chain length of polyprenol and dolichol in the leaf samples derived from in vitro propagation of the *Pisifera* S8 and S24 parents.

Keywords: Dolichol, oil palm, polyprenol, tissue culture

INTRODUCTION

The African oil palm (*Elaeis guineensis* Jacq.) is a tropical plantation crop cultivated for its production of two types of vegetable oil: palm oil and palm kernel oil. It is a monocotyledonous species belonging to the family of Areaceae, which includes more than 2,000 palm species (Hormaza et al. 2012). In recent decades, palm oil has become an important commodity as a source of world vegetable oil for many industries. For more than a decade, the palm oil sector in Indonesia has experienced massive growth, between 1990 and 2013 the area used for oil palm plantations increased tenfold from 1.1 million hectares to 10.4 million hectares with a total production of CPO (crude palm oil) 27.7 million tons of raw material, which is nearly half of global production (Yudawinata 2017).

The increasing market demand for oil palm seeds has initiated seed producers to meet the needs of oil palm seedlings through in vitro plant propagation or tissue culture approaches. Oil palm is typically propagated in vitro by indirect somatic embryogenesis, a process in which somatic cells from selected explants are induced to

differentiate into somatic embryos through an intermediate phase, namely callus formation. The existence of oil palms that do not have axillary shoots, making it impossible for vegetative propagation to be done by micro cuttings (Jayanthi et al. 2011). The embryogenic callus structure is extensively propagated to increase the efficiency of somatic embryos, but this stage risks increasing the frequency of somaclonal variations (Rival et al. 2013). Somaclonal variation is defined as the genetic diversity of plants produced through the cell and plant tissue culture, both from somatic cells such as leaves, roots and stems as well as gamete cells (Neumann et al. 2017).

Plants that are produced from somaclonal variations in vitro can change their properties differ from the original plant, either permanently or temporarily. Temporary changes in traits result from epigenetic or physiological influences and are not heritable and can return to the original plant characteristics (Kaeppeler et al. 2000; Miguel and Marum 2011). Meanwhile, permanent change in the traits was defined as somaclonal diversity that can be inherited and express the diversity that is owned by early plants, even giving rise to new diversity caused by

unexplained genetic mechanisms (Smulders and De Klerk 2011; Mgbeze and Iserhienrhien 2014).

One of the secondary metabolites that are abundant in oil palm is a polyisoprenoid alcohol compound. Polyisoprenoids are metabolites that play a role in plant defense systems against biotic and abiotic stresses (Basyuni et al. 2017a; Baczewska et al. 2014). Two main groups of polyisoprenoids are polyprenol and dolichol that can be distinguished by the presence of an unsaturated (polyprenol) or saturated (dolichol) α -isoprene subunit (Basyuni et al. 2016; Chang et al. 2015; Surmacz and Swiezewska 2011). Polyprenol typically ranges in size from C45-C60 as shorter polyprenol, medium (C65-C85), and longer polyprenol (C90-C140). Dolichol is present in nearly all plant tissues, particularly in the roots (Basyuni et al. 2016, 2017a, b, 2018a), from the shorter (C25-C40), medium-chain (C65-C85), and longer chain length (C90-C140). In Arifiyanto et al. (2017) research, the length of the polyisoprenoid carbon chain in oil palms varies according to each tissue or organ even though it is in the same species but comes from different growth phases and the type of fruit produced.

Several studies have been reported that the profile and occurrence of polyisoprenoids can be used as chemotaxonomic markers (Basyuni et al. 2016, 2017b, 2018b; Arifiyanto et al. 2017). The results of this study explain the differences in expression on the type, content, and carbon chain length of the polyisoprenoid from each individual in vitro culture as an indicator of somaclonal variation. Furthermore, this study made it possible to classify individuals in one stable clone from the same parent, in vitro stages and in the same culture environment. Therefore, this study aims to analyze the presence of somaclonal variations that apply to oil palm individuals resulting from tissue culture based on the presence of polyisoprenoid compounds used as chemotaxonomic markers.

MATERIALS AND METHODS

Study area

The plant material was collected from two plantation locations of PT. Socfin Indonesia, namely two samples of leaves of *Pisifera* parents (S8 and S24) obtained from Kebun Dolok Masihul, Serdang Bedagai District, North Sumatra, Indonesia; and 30 (thirty) leaf samples respectively from in vitro propagation of the S8 and S24 parents were obtained from Aek Loba Plantation, Asahan District, North Sumatra, Indonesia, in February 2020. Extraction and analysis of polyisoprenoid content were carried out at the Forestry Biotechnology Laboratory, Faculty of Forestry, University of North Sumatra, Medan, in March-December 2020.

Chemicals

The dolichol (C90-C105) and polyprenol (C90-C100) standards were used as previously reported (Basyuni et al. 2016, 2017a) to identify the pattern of polyisoprenoid alcohol in the plant materials. The solvents and chemicals

used, such as chloroform, methanol, ethanol, hexane, toluene, ethyl acetate, KOH are chemicals with analytical grade quality (Merck®). For the separation of chemical compounds are used Silica gel 60 TLC plate and RP-18 reverse-phase silica HPTLC plate (Merck®).

Isolation of polyprenol and dolichol

The isolation of polyprenol and dolichol compounds was carried out as described previously in Basyuni et al. (2016, 2017a, c) and Arifiyanto et al. (2017). Oil palm leaf samples for each number were dried at 60-75°C for 3 days. The dried leaf samples were crushed into a fine powder using a mill. For each sample, individual numbers were weighed as much as 5 g and immersed in chloroform/methanol (2:1), then incubated in a water bath for 48 hours. Furthermore, the extract was filtered to produce a supernatant using filter paper No. 2 (Advantec®). The supernatant was dried using a rotary evaporator to produce dry fat. Then weighed to obtain lipid weight and lipid percentage to dry weight of leaf samples (mg g^{-1}).

For all individual numbers of samples, the saponification of dry lipid extract was carried out in 86% ethanol solvent containing 2 M KOH, at 65 °C for 24 hours. The unsaponified lipids are evaporated and redissolved in the hexane solvent. Then a two-dimensional thin-layer chromatography (TLC) analysis was performed using 100 mg of the sample for each number (Basyuni et al. 2018a; 2019).

The first dimensional TLC was carried out on a silica gel glass (20x3 cm) using toluene: ethyl acetate (9:1) developing solvent for 45 minutes (Basyuni et al. 2016). The longitudinal edge of the first dimensional TLC and the concentration zone of reverse phase TLC C-18 are connected by a magnetic bar clamp. The silica gel glass and C-18 of TLC plate were then developed in acetone for about 30 minutes to transfer all compounds that have been separated in the first dimension into the concentration zone of the reverse-phase TLC plate. Polyprenol and dolichol standards were also developed on silica gel glass and C-18 of TLC plates along with the sample lines using the development solvent system described previously. The use of this standard aims to confirm the presence of both compounds and also to determine the polyprenol and dolichol pattern from each sample analyzed based on the length of the carbon (C) chain in the detected compound. The presence of polyisoprenoid alcohol in the sample is characterized by the appearance of spots on the C18 of TLC plates developed with a solvent system and can be seen with iodine vapor. The results of this 2D-TLC chromatography can be scanned using the Canon G2000. The polyisoprenoid pattern of the sample was determined by comparing dolichol or/and polyprenol standards on TLC plates.

A standard curve constructed from the authentic standard dolichol and polyprenol is used to determine the content of the polyisoprenoid family in samples via correlation of mobility on TLC using iodine staining detection in the second-dimensional phase. Dolichol and polyprenol content in the sample was determined using ImageJ ver. 1.46r (Schneider et al. 1992), was further

compared with the standard curves (dolichol and standard polyprenol) drawn previously.

Cluster analysis

Cluster analysis was performed on a selected subset of data from the carbon chain length of polyisoprenoids, together with the polyprenol and dolichol content (24 variables) of the leaves of the S8 and S24 Pisifera samples (parents), and 30 (thirty) leaf samples of propagated plants in vitro of the Pisifera S8 and S24 respectively. The data obtained was transformed in log (10) form as previously reported (Basyuni et al. 2018b). Dendrogram representing carbon chain length data for all samples drawn by clustering analysis using the Un-weighted Pair Group Method with Arithmetic mean (UPGMA) in MVSP (Multivariate Statistical Package) 3.22 Software (Kovach Computing Service). Euclidean distance is set as an indicator for the combination of the cluster.

RESULTS AND DISCUSSION

Polyisoprenoid in Pisifera parent oil palm at PT Socfin Indonesia, Dolok Masihul Estate

Based on the results of extraction, saponification, and analysis of polyisoprenoid content using two-dimensional Thin Layer Chromatography (TLC) on the parent plants of Pisifera S8 and S24 oil palms in Kebun Dolok Masihul PT. Socfin Indonesia shows a different quantity for each metabolite content analyzed in Table 1. Leaf samples from parent S8 showed lower total lipid (7.40 mg g⁻¹ dry weight) and polyprenol content (0.67 mg g⁻¹) than parent S24 (8.90 mg g⁻¹ dry weight for total lipid, and 1.45 mg g⁻¹ polyprenol). However, for dolichol content, the S8 parents showed a higher content (1.5 mg g⁻¹) compared to the S24 parents (0.95 mg g⁻¹). The largest number of polyisoprenoid was found in the S24 leaf sample of 2.41 mg g⁻¹ dry weight while the smallest number of polyisoprenoid was in the S8 leaf sample of 2.17 mg g⁻¹ dry weight.

Table 1. Total lipid, polyprenol, and dolichol profile in leaves of the parent plants of Pisifera S8 and S24 oil palms

Elder	TL (mg g ⁻¹ dw)	PI (mg g ⁻¹ dw)	Pol (mg g ⁻¹)	Dol (mg g ⁻¹)	% in Total lipid			% in polyisoprenoid		Type
					PI	Pol	Dol	Pol	Dol	
S8	7.40	2.17	0.67	1.50	29.32	9.05	20.27	30.88	69.12	II
S24	8.90	2.41	1.45	0.95	27.08	16.29	10.67	60.17	39.42	II

Note; TL: total lipid, PI: polyisoprenoids, Pol: polyprenol, Dol: dolichol, dw: dry weight

Table 2. Total lipid, polyprenol, and dolichol profile in the leaves of oil palm plants as a result of tissue culture propagation from Pisifera S8

No. individual	TL (mg g ⁻¹ dw)	PI (mg g ⁻¹ dw)	Pol (mg g ⁻¹)	Dol (mg g ⁻¹)	% in Total lipid			% in Polyisoprenoid		Type
					PI	Pol	Dol	Pol	Dol	
S8 93/1	61.00	7.92	4.42	3.50	12.99	7.26	5.74	55.83	44.16	II
S8 93/2	58.70	8.53	5.00	3.52	14.53	8.53	6.00	58.71	41.28	II
S8 93/3	91.60	7.81	4.28	3.52	8.53	4.68	3.85	54.89	45.10	II
S8 93/4	47.50	2.92	1.90	1.01	6.16	4.02	2.14	65.25	34.74	II
S8 93/5	56.20	1.95	0.97	0.97	3.47	1.74	1.73	50.09	49.90	II
S8 93/6	74.90	2.63	1.14	1.48	3.51	1.53	1.98	43.56	56.43	II
S8 93/7	53.60	3.88	2.12	1.75	7.24	3.97	3.27	54.81	45.18	II
S8 93/8	60.70	2.21	0.96	1.25	3.66	1.59	2.06	43.62	56.37	II
S8 93/9	42.30	3.25	1.82	1.43	7.69	4.31	3.38	56.01	43.98	II
S8 93/10	74.50	1.54	0.49	1.04	2.06	6.60	1.40	32.01	67.98	II
S8 93/11	54.40	8.32	4.39	3.92	1.53	8.09	7.22	52.83	47.16	II
S8 93/12	58.00	2.94	1.47	1.44	5.07	2.55	2.49	50.29	49.10	II
S8 93/13	73.90	3.00	1.06	1.93	4.06	1.45	2.62	35.56	64.43	II
S8 93/14	62.00	1.89	1.35	0.53	3.06	2.19	8.71	71.55	28.44	II
S8 93/15	56.60	3.57	1.91	1.65	6.32	3.39	2.93	53.63	46.36	II
S8 93/16	72.80	3.14	1.70	1.44	4.32	2.33	1.98	54.09	45.90	II
S8 93/17	61.70	1.71	0.90	0.81	2.78	1.46	1.32	52.60	47.39	II
S8 93/18	58.70	3.48	1.96	1.52	5.94	3.35	2.59	56.37	43.62	II
S8 93/19	63.30	1.82	0.61	1.21	2.88	9.67	1.91	33.57	66.42	II
S8 93/20	46.30	1.93	0.65	1.28	4.18	1.40	2.77	33.68	66.31	II
S8 94/11	64.40	3.42	1.79	1.63	5.32	2.79	2.54	52.43	47.56	II
S8 94/12	68.80	4.30	2.31	1.98	6.26	3.36	2.89	53.77	46.22	II
S8 94/14	59.40	2.61	1.13	1.46	4.39	1.91	2.47	43.59	56.40	II
S8 94/15	99.60	2.29	0.72	1.56	2.31	7.31	1.58	31.68	68.31	II
S8 94/16	54.80	1.29	0.46	0.82	2.36	8.52	1.51	36.08	63.91	II
S8 94/17	76.30	1.37	0.81	0.55	1.79	1.07	7.24	59.67	40.32	II
S8 94/18	54.40	2.29	1.10	1.18	4.22	2.04	2.18	48.36	51.63	II
S8 94/20	30.80	7.28	4.11	3.17	23.65	13.35	10.30	56.46	43.53	II
S8 94/21	56.30	2.94	1.99	0.93	5.22	3.55	1.66	68.08	31.91	II
S8 94/22	51.50	0.71	0.18	0.53	1.38	3.46	1.04	25.03	74.96	II

Note; TL: total lipid, PI: polyisoprenoids, Pol: polyprenol, Dol: dolichol, dw: dry weight

Table 3. Total lipid, polyprenol, and dolichol profile in the leaves of oil palm plants as a result of tissue culture propagation from *Pisifera* S24

No. individual	TL (mg g ⁻¹ dw)	PI (mg g ⁻¹ dw)	Pol (mg g ⁻¹)	Dol (mg g ⁻¹)	% in total lipid			% in polyisoprenoid		Type
					PI	Pol	Dol	Pol	Dol	
S24 H1	65.40	0.96	0.45	0.51	1.48	0.69	0.79	46.96	53.03	II
S24 H2	66.00	1.76	0.80	0.95	2.68	1.23	1.45	45.83	54.16	II
S24 H3	12.40	1.12	0.47	0.65	9.10	3.83	5.28	42.05	57.94	II
S24 H4	71.80	3.27	1.91	1.35	4.57	2.67	1.88	58.43	41.25	II
S24 H5	48.30	6.71	3.59	3.11	13.90	7.45	6.45	53.58	46.41	II
S24 H6	81.00	1.26	0.98	0.27	1.57	1.22	0.34	78.00	21.99	II
S24 H7	99.90	7.72	2.50	5.22	7.74	2.50	5.23	32.34	67.65	II
S24 H8	16.00	2.11	0.79	1.31	13.19	4.99	8.20	37.82	62.17	II
S24 H9	60.90	1.73	0.81	0.91	2.85	1.34	1.51	47.13	52.86	II
S24 H10	65.70	4.48	2.42	2.06	6.83	3.69	3.15	53.97	46.02	II
S24 H11	32.10	1.54	1.11	0.42	4.80	3.46	1.34	72.10	27.89	II
S24 H12	90.00	1.56	0.61	0.95	1.74	6.78	1.06	38.94	61.05	II
S24 H14	32.10	0.73	0.51	0.22	2.28	1.59	0.69	69.78	30.21	II
S24 H15	58.40	1.38	0.70	0.68	2.37	1.20	1.17	50.60	49.39	II
S24 H16	114.80	8.05	3.26	4.79	7.02	2.84	4.17	40.51	59.48	II
S24 H17	41.80	1.43	0.57	0.86	3.43	1.37	2.06	39.86	60.13	II
S24 H18	45.40	1.13	0.65	0.48	2.51	1.44	1.07	57.32	42.67	II
S24 H19	71.70	2.27	0.98	1.29	3.17	1.37	1.80	43.14	56.85	II
S24 H20	14.00	1.25	0.41	0.83	8.95	2.99	5.96	33.37	66.62	II
S24 H21	139.00	3.45	1.63	1.81	2.48	1.18	1.31	47.32	52.67	II
S24 H22	74.80	3.48	1.86	1.61	4.66	2.50	2.17	53.57	46.42	II
S24 H23	45.20	1.60	0.65	0.94	3.54	1.45	2.09	40.87	59.12	II
S24 H24	68.80	1.64	0.69	0.94	2.38	1.02	1.37	42.65	57.34	II
S24 H25	74.10	1.38	0.64	0.74	1.87	0.88	0.99	46.69	53.30	II
S24 H26	113.00	1.63	0.86	0.76	1.45	0.77	0.68	53.09	46.90	II
S24 H27	64.30	2.45	1.13	1.32	3.82	1.76	2.06	46.05	53.94	II
S24 H28	82.90	0.95	0.36	0.58	1.15	0.45	0.71	38.67	61.32	II
S24 H29	60.30	2.58	2.12	0.45	4.28	3.52	0.76	82.22	17.77	II
S24 H30	173.1	1.71	1.03	0.68	0.99	0.60	0.39	60.39	39.60	II
S24 H31	65.00	1.71	1.13	0.58	2.64	1.74	0.90	65.96	34.03	II

Note; TL: total lipid, PI: polyisoprenoids, Pol: polyprenol, Dol: dolichol, dw: dry weight

The composition of polyprenol and dolichol is divided into three types, namely types I, II, and III (Basyuni 2016). Type I, if there is more than 90% dominance of dolichol; Type II, shows a balanced distribution of polyprenol with dolichol; Type III, the dominance of polyprenol compounds is 90% in plant tissue. Leaf samples from the *Pisifera* mother tree found at PT. Socfin Indonesia is classified as type II. The results of previous studies also indicated that leaf samples from sprouts and adult plants of Tenera (Afriyanto et al. 2017), as well as leaf and mesocarp samples from 90 tested progeny (Habsyah et al. 2021), were included in the polyisoprenoid type II distribution. This type of pattern becomes a chemotaxonomic marker for oil palm plants in agro-climatic conditions similar to the location of the research activity.

Polyisoprenoids from tissue culture plants in Aek Loba Estate

Analysis of polyisoprenoid leaf samples from tissue culture plants sourced from *Pisifera* S8 and S24 parents Aek Loba Garden showed a varied distribution of polyprenol and dolichol. Table 2 and Table 3 showed the distribution of polyprenol and dolichol in the in vitro yield plants of *Pisifera* S8 and S24 parents, respectively.

Total lipids in the in vitro propagation results of *Pisifera* S8 derivatives ranged from 30.80 – 99.60 mg g⁻¹ dry weight with the lowest total lipids found in S8 94/20 and the highest total lipids found in cultivar S8 94/15. Meanwhile, the total lipids found in vitro propagation plants results derived from *Pisifera* S24 ranged from 12.40 - 173.1 mg g⁻¹ dry weight with the lowest total lipids in cultivar S24H3 and the highest total lipids in cultivar S24H30. For polyisoprenoid values in the S8 plants ranged from 0.71 - 8.53 mg g⁻¹ dry weight was found in S8 94/22 and S8 93/2, while for polyisoprenoids in the S24 *Pisifera* derivatives ranged from 0.73 - 8.05 mg g⁻¹ dry weight. Based on the data above, it can be seen that the oil palm samplings of *Pisifera* S8 and S24 types are type II, where the individual cultivars contain proportional amounts of polyprenol and dolichol in plant tissue (Basyuni et al. 2016). Type II in the plant tissue culture results showed similarities to the parent plant source S8 and S24 as a source of explants where the parent plant also contained nearly the same proportion of polyprenol and dolichol. Based on the type of composition of the polyisoprenoid content, there was no difference between parent plants and tillers, both for type S8 and S24.

Table 4. Carbon (C) chain length polyprenols and dolichol from oil palm leaves of two Pisifera parents at PT. Socfin Indonesia, Dolok Masihul Estate, Serdang Bedagai District, North Sumatra, Indonesia

Elder of Pisifera	Polyprenol	Dolichol
S8	75 80 85	75 80 100 105 110
S24	45 50 55 60 65	50 55 90 95 100

Table 5. Carbon chain length (C) polyprenol and dolichol from the oil palm leaves propagated by tissue culture from Pisifera S8 at PT Socfin Indonesia, Kebun Aek Loba, Asahan District, North Sumatra, Indonesia

No. individual	Polyprenol	Dolichol
S8 93/1	45 50 55 60 65 70 85 90 95	50 55 60 80 85 90 95 100
S8 93/2	45 50 55 60 65 70 85 90 95	50 55 60 80 85 90 95 100
S8 93/3	45 50 55 60 65 70 85 90 95	50 55 60 85 90 95 100
S8 93/4	45 50 55 60 65 70 75	50 55 60 85 90 95 100 105 110
S8 93/5	45 50 55 60 65	50 55 80 85 90 95
S8 93/6	45 50 55 60 65	50 55 60 65 80 85 90 95 100 105
S8 93/7	45 50 55 60 65	55 60 65 85 90 95 100
S8 93/8	45 50 55 60 65	50 55 60 90 95 100 105
S8 93/9	50 55 60	50 55
S8 93/10	45 50 55 60 65	50 55 60 90 95 100 105
S8 93/11	45 50 55 60 65	50 55 60 85 90 95 100
S8 93/12	45 50 55 60 65	50 55 60 85 90 95 100
S8 93/13	45 50 55 60 65	50 55 60 85 90 95 100
S8 93/14	50 55 60	50 55 85 90 95 100
S8 93/15	45 50 55 60 65	55 60 65 85 90 95 100
S8 93/16	45 50 55 60 65	90 95
S8 93/17	45 50 55 60 65 70	90 95 100 105
S8 93/18	45 50 55 60 65	50 55 60 85 90 95 100
S8 93/19	45 50 55 60 65	50 55 60 90 95 100 105
S8 93/20	45 50 55 60 65 70	50 55 60 90 95 100
S8 94/11	45 50 55 60 65	50 55 60 85 90 95 100
S8 94/12	50 55 60	50 55 60 65
S8 94/14	45 50 55 60 65	50 55 60 85 90 95 100
S8 94/15	50 55	50 55 60 85 90 95 100
S8 94/16	45 50 55 60 65	50 55 60 85 90 95 100
S8 94/17	80 85 90 95	90 95
S8 94/18	45 50 55 60 65	50 55 90 95 100 105
S8 94/20	45 50 55 60 65	50 55 60 85 90 95 100
S8 94/21	45 50 55 60 65	85 90 95
S8 94/22	45 50 55 60	50 55 60 85 90 95 100

Carbon chain length analysis

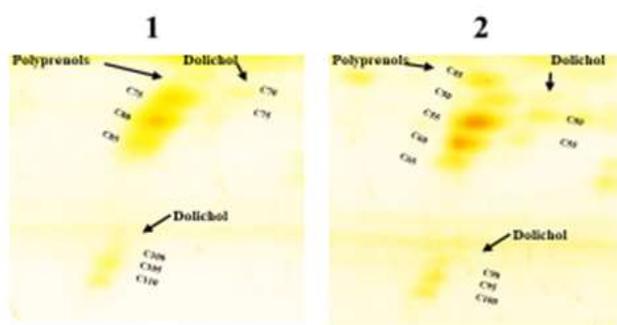
Polyisoprenoid compounds from oil palm leaves which are grouped into polyprenol and dolichol can be identified and distinguished by their carbon chain length using the two-dimensional Thin Layer Chromatography Analysis method (Sagami et al. 1992, Basyuni et al. 2016). Previously, Arifiyanto et al. (2017) reported that the presence of polyprenol and dolichol in Tenera hybrid of palm oil fruit mesocarp and fruit shell from Lame.

The results of the study obtained explain that parent numbers of S8 and S24 as the Pisifera type of oil palm plant, in the Dolok Masihul Plantation of PT Socfin Indonesia was classified as Type II, where the distribution of polyprenol and dolichol in plant tissue was almost the same proportion. The length of the polyprenol carbon chain on S8: C75 - C85, and dolichol: C75 - 110, while the carbon polyprenol chain on S24: C45 - C65, and dolichol: C50 - C100 (Table 4 and Figure 1). The difference in the length of the polyisoprenoids carbon chain in the two samples from the Pisifera parent was caused by several factors,

including increasing plant age, salinity stress, and tissue differences (Basyuni et al. 2014). Polyisoprenoid is a secondary metabolite of the terpenoid class, a related biochemical enhancer in the plant defense system against biotic and abiotic disorders (Basyuni et al. 2019). In Arifiyanto et al. (2017), the average length of polyisoprenoid carbon varies according to their respective tissues even though they are in the same species and form certain dominant families. Dolichol act as sugar-carrying lipids in N-glycoprotein biosynthesis. Dolichol has a clear role as a lipid carrier for glycan precursors in the early stages of glycosylation of N-linked proteins, which accumulate in the endoplasmic reticulum of all eukaryotic cells (Cantagrel and Lefebvre 2011). Tateyama et al. (1999) also stated that the carbon chain length distribution of polyprenol is not necessarily the same as the chain length of carbon dolichol in the same tissue. Plant polyprenol accumulated in the chloroplast determines the fluidity of thylakoid membrane which affects the efficiency of photosynthesis (Tarik et al. 2017).

Table 6. Carbon chain length (C) polyprenol and dolichol from oil palm leaves propagated by tissue culture from Pisifera S8 at PT Socfin Indonesia, Dolok Masihul Estate, Asahan District, North Sumatra, Indonesia

No. individual	Polyprenol	Dolichol
S24 H1	45 50 55 60	50 55 60 85 90 95
S24 H2	45 50 55 60 65	50 55 60 90 95 100
S24 H3	45 50 55 60 65	50 55 60 85 90 95 100
S24 H4	45 50 55 60 65 70	55 60 90 95 100 105
S24 H5	45 50 55 60 65 70	55 60 85 90 95 100
S24 H6	45 50 55 60 65	50 55 60 80 85 90 95
S24 H7	50 55 60 65	45 50 55
S24 H8	45 50 55 60 65	50 55 60 90 95 100
S24 H9	45 50 55 60 65	50 55 60 65 90 95 100
S24 H10	45 50 55 60 65	50 55 60 85 90 95
S24 H11	45 50 55 60 65	50 55 80 85 90 95
S24 H12	45 50 55 60 65	50 55 60 85 90 95 100
S24 H14	50 55 60 65	85 90 95 100
S24 H15	50 55 60 65 90 95	50 55 85 90 95 100
S24 H16	45 50 55 60 65 70 90 95	45 50 55 60 85 90 95 100
S24 H17	45 50 55 60 65	50 55 60 85 90 95 100 105
S24 H18	50 55 60 65	85 90 95 100
S24 H19	45 50 55 60 65	50 55 60 90 95 100 105
S24 H20	45 50 55 60 65	50 55 60 95 100 105
S24 H21	45 50 55 60 65	50 55 60 95 100 105
S24 H22	45 50 55 60 65 80 85 90	50 55 60 85 90 95 100
S24 H23	45 50 55 60 65	50 55 60 85 90 95
S24 H24	45 50 55 60 65 70	50 55 60 65 90 95 100 105
S24 H25	45 50 55 60 65 70	50 55 60 85 90 95 100 105
S24 H26	45 50 55 60 65 70	90 95 100 105
S24 H27	45 50 55 60 65 70	55 60 65 95 100 105
S24 H28	45 50 55 60 65	50 55 60 90 95 100
S24 H29	45 50 55 60 65	85 90 95
S24 H30	45 50 55 60 65 70 90 95 100	55 60 65 90 95 100 105
S24 H31	45 50 55 60 65	85 90 95 100

**Figure 1.** Two-dimensional TLC of polyisoprenoid compounds of oil palm leaf tissue of Pisifera S8 (1) and S24 (2) parents obtained from PT Socfin Indonesia, Dolok Masihul Estate, Serdang Bedagai District, North Sumatra, Indonesia

Tables 5 and 6 show that dolichol and polyprenol also dominate the polyisoprenoids in oil palm leaves produced by in vitro propagation of the Pisifera parent. The shortest carbon chain length in plant tissue culture from Pisifera S8 parent, namely for polyprenol with the carbon chain length of C50-C55 found in S8 94/15, and the longest found in S8

93/1, S8 93/2, and S8 93/3, namely C45-C95. While the shortest carbon chain length of dolichol was found in the individual number of S8 93/9, namely C50-C55, and the longest carbon chains were obtained in the individual number of S8 93/4, namely C50-C110 (Table 5, Figure 2 and Figure 3). The carbon chain lengths of the polyprenol and dolichol from the tissue culture propagated plants are different from their parent plants. The type of chain carbon length of polyprenol found in the S8 parents (C75, C80, and C85) was also found in the plant tissue culture using S8 as the parent, namely S8 93/1, S8 93/2, S8 93/3, S8 93/4, and S8 94/17. However, the composition of the carbon chain length of the polyprenol group of tissue culture propagation plants has a more varied composition of carbon chain lengths (between C45 - C90) for each number of individual and is not shared by the parent S8, especially the carbon chain length of the C45-C70. For the dolichol group, the individual plants from tissue culture propagation showed the type of dolichol carbon chain length that belonged to the Pisifera S8 parent, except for a few individuals who did not have the carbon chain length of parent S8, namely, S8 93/9, S8 93/16, S8 94/12, S8 94/17 and S8 94/21 (Table 5, Figures 2 and 3).

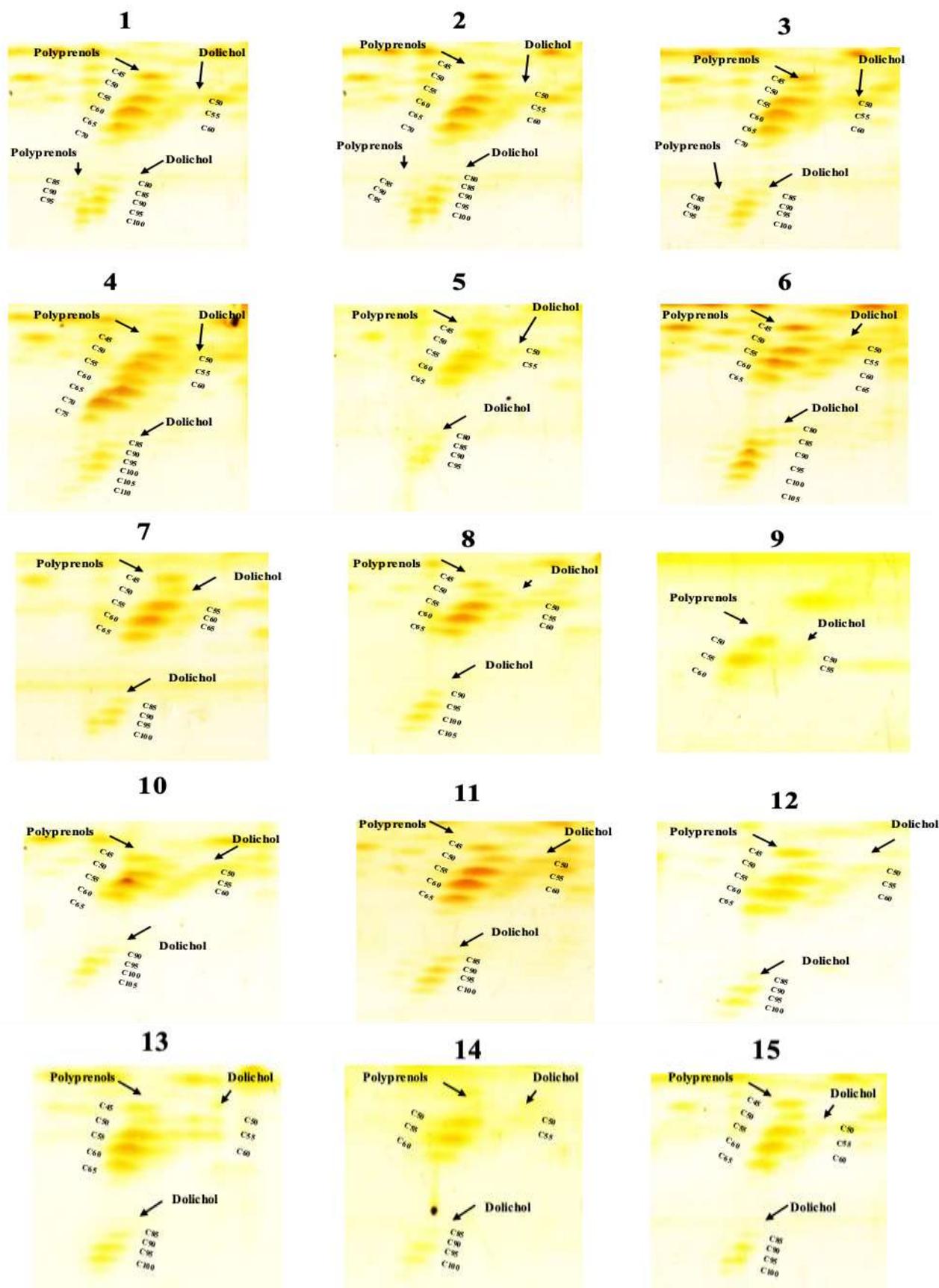


Figure 2. Two-dimensional TLC of polyisoprenoid compounds from oil palm leaf tissue derived from in vitro propagated individuals of *Pisifera* S8 parents obtained from PT Socfin Indonesia, Kebun Aek Loba, Asahan District, North Sumatra, Indonesia. No. Individual S8 93/1 (1), S8 93/2 (2), S8 93/3 (3), S8 93/4 (4), S8 93/5 (5), S8 93/6 (6), S8 93/7 (7), S8 93/8 (8), S8 93/9 (9), S8 93/10 (10), S8 93/11 (11), S8 93/12(12), S8 93/13 (13), S8 93/14 (14), and S8 93/15 (15)

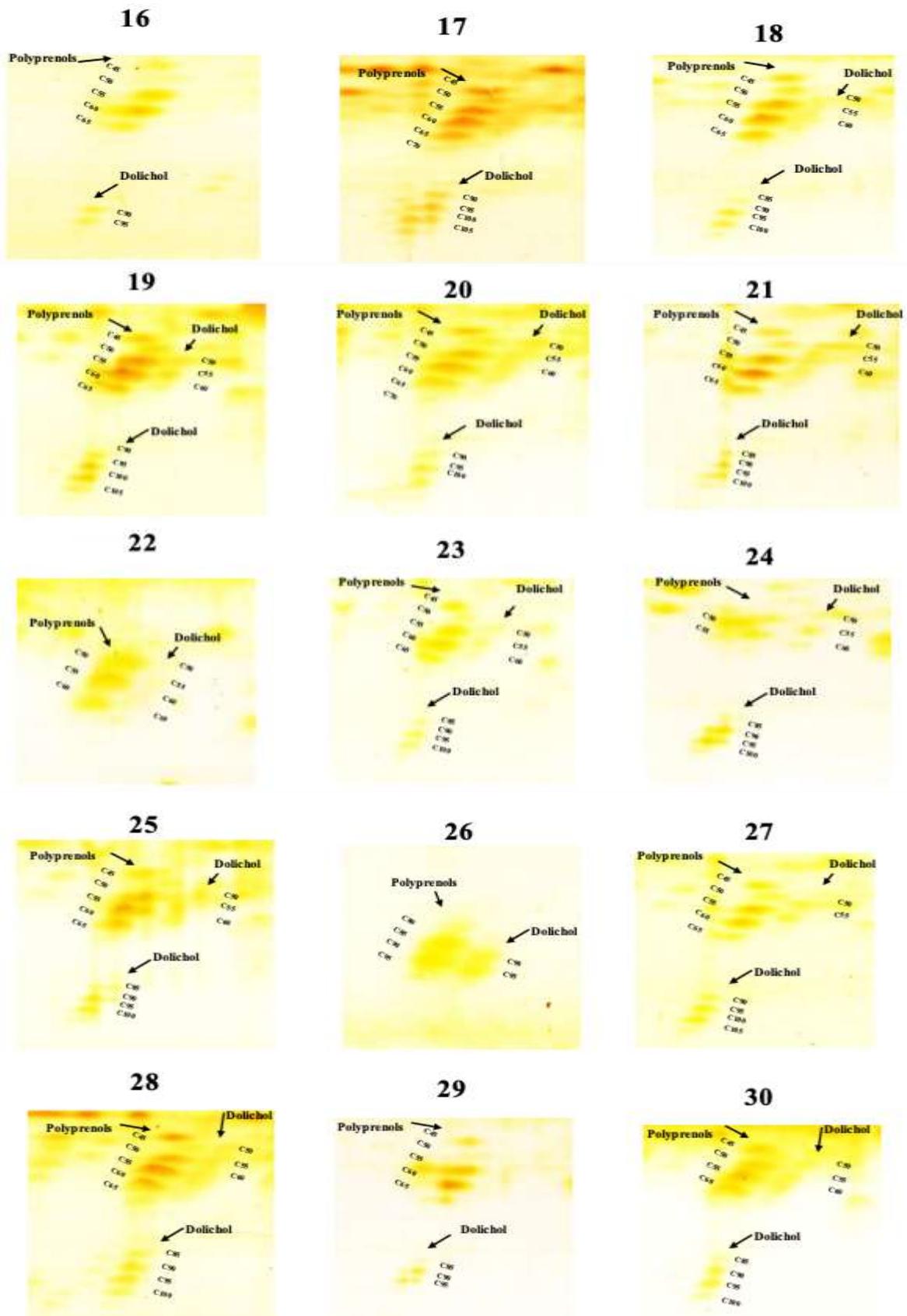


Figure 3. Two-dimensional TLC of polyisoprenoid compounds from oil palm leaf tissue derived from in vitro propagated individuals of *Pisifera* S8 parents obtained from PT Socfin Indonesia, Kebun Aek Loba, Asahan District, North Sumatra, Indonesia. No. Individual S8 93/16 (16), S8 93/17(17), S8 93/18(18), S8 93/19 (19), S8 93/20 (20), S8 94/11 (21), S8 94/12 (22), S8 94/14 (23), S8 94/15 (24), S8 94/16(25), S8 94/17 (26), S8 94/18 (27), S8 94/20 (28), S8 94/21 (29), and S8 94/22 (30).

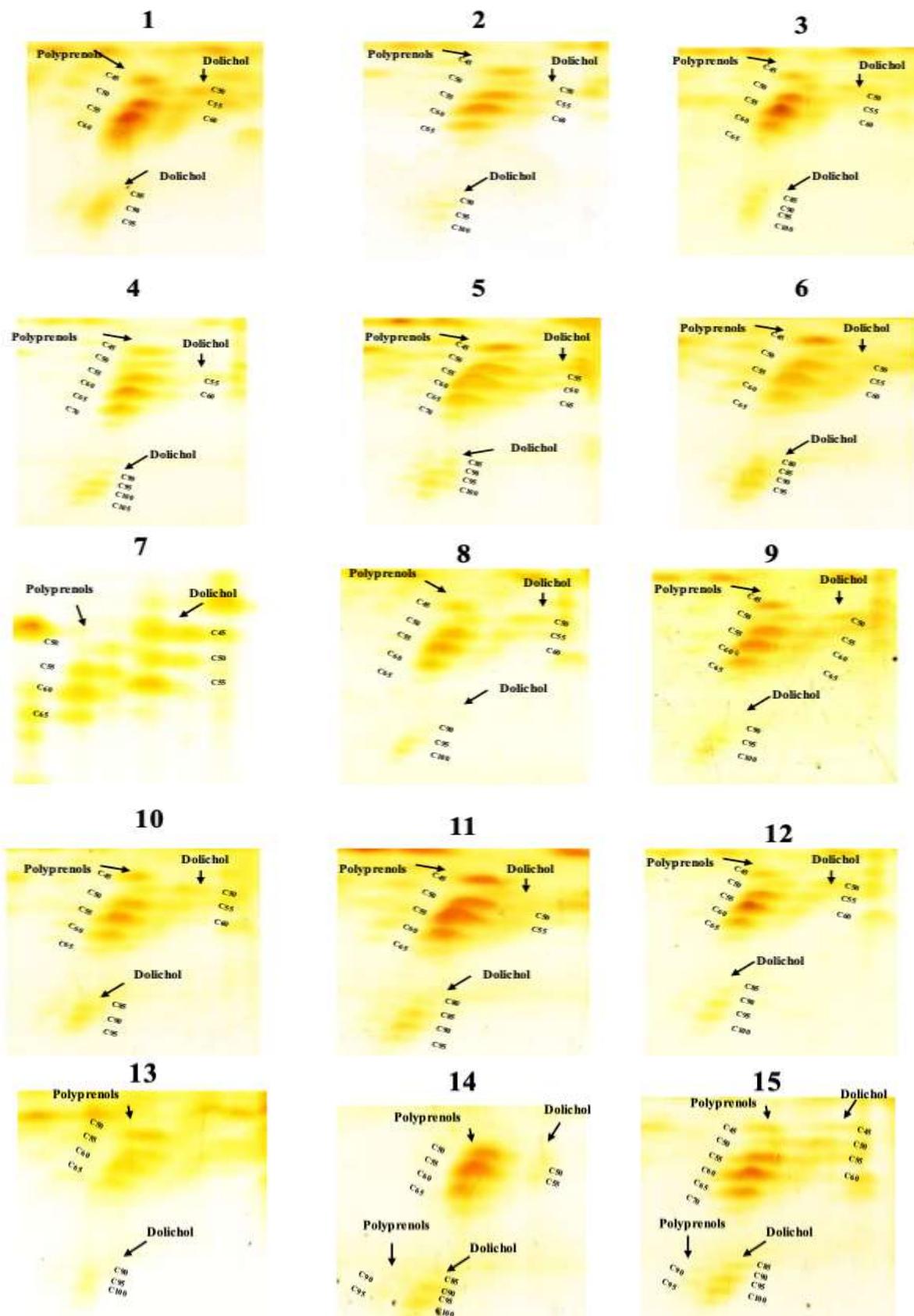


Figure 4. Two-dimensional TLC of polyisoprenoid compounds from oil palm leaf tissue derived from in vitro propagated individuals of *Pisifera* S24 parents obtained from PT Socfin Indonesia, Kebun Aek Loba, Asahan District, North Sumatra, Indonesia. No. Individual S24 H1 (1), S24 H2 (2), S24 H3 (3), S24 H4 (4), S24 H5 (5), S24 H6 (6), S24 H1 (7), S24 H8 (8), S24 H9 (9), S24 H10 (10), S24 H11 (11), S24 H12 (12), S24 H14 (13), S24 H15 (14), dan S24 H16 (15)

For samples of in vitro plant propagation from Pisifera S24 parent, the shortest polyprenol carbon chain was obtained in the individual number of S24 H1, namely C45-C60, and the longest found in the individual number of S24 H30, namely C45-C100, while for the shortest dolichol obtained from an individual number of S24H7, namely C45-C55, while other individuals have the same average carbon chain, namely C50-C105 (Table 6, Figure 4 and Figure 5). For the individual numbers from the in vitro propagation of parent S24, all of them indicated the presence of one or more of the carbon polyprenol belonging to the parent S24 (C45, C50, C55, C60, and C65), in addition to several individual clones has a carbon chain length that is not owned by the parent S24. Whereas in the plant tissue culture of the S24 parent, all of them had one or several types of carbon chain lengths that were also owned by the S24 parent, and some of the in vitro propagated individuals had the type of carbon chain lengths that the S24 parent did not (Table 6, Figure 4 and Figure 5).

Dolichol is the main polyisoprenoid compound in many flowering plants, and only a few polyprenol can be found (Sagami et al. 1992; Basyuni et al. 2017b). In the cell of eukaryotic, *cis*-prenyltransferases (CPTs) produce prenyl diphosphate that in some tissues is converted to dolichol due to the activity of polyprenol reductase (Cantagrel et al. 2010; Jozwiak et al. 2015). The prenyl diphosphate biosynthetic pathway in the eukaryotic cell requires the interaction of CPTs with an accessory CPT-binding protein for the determination of polyprenol chain length, and the examples of such complexes were found at some plants, i.e. tomato (Brasher et al. 2015), lettuce (Qu et al. 2015), Arabidopsis (Kwon et al. 2016), and guayule (Lakusta et al. 2019). The Arabidopsis genome contains nine putative *cis*-prenyltransferase encoding genes (AtCPT1 to AtCPT9) that

are expressed in a tissue-specific manner. AtCPT1, -6, and -9 are exclusively expressed in roots, and simultaneously several dolichols are identified in this tissue (Surmacz et al. 2011; Jozwiak et al. 2013).

Each plant in vitro still showed the same polyisoprenoid compound group as the Pisifera S8 and S24 parents. However, some of the in vitro propagated individuals showed the presence of polyprenol and dolichol which had different numbers and types of carbon chain lengths from the S8 and S24 parents. The question is whether the diversity of the number of carbon chain lengths possessed by individuals from tissue culture has occurred since the morphogenesis/embryogenesis process during in vitro propagation, or whether this diversity occurs after the plants are moved to the field as a result of various stresses in environmental factors that affect the expression of the presence of polyisoprenoid. Biomass from the *Taxus baccata* cell suspension culture showed changes in the content of isoprenoid alcohol (polyprenol with very low dolichol content) due to differences in culture age and addition of elicitor in the culture medium but did not affect the dominance of polyprenols with carbon chain lengths of C17 and C22. Meanwhile, in hairy root culture from *Taxus media*, the isoprenoid alcohol content was dominated by dolichol with carbon chain lengths C17 and C22. (Skorupinska-Tudek et al. 2007). It is noteworthy that polyprenol and dolichol level in leaves tissue of mature plant was higher than that of the seedlings. Increased polyprenol in leaves with increasing age has also been reported in *Kandelia obovate* and *Bruguiera gymnorrhiza* yellow leaf (Basyuni et al. 2016), old leaves of ginkgo and old rubber leaf (Tateyama et al. 1999), and senescing leaves (Swiezewska et al. 1994).

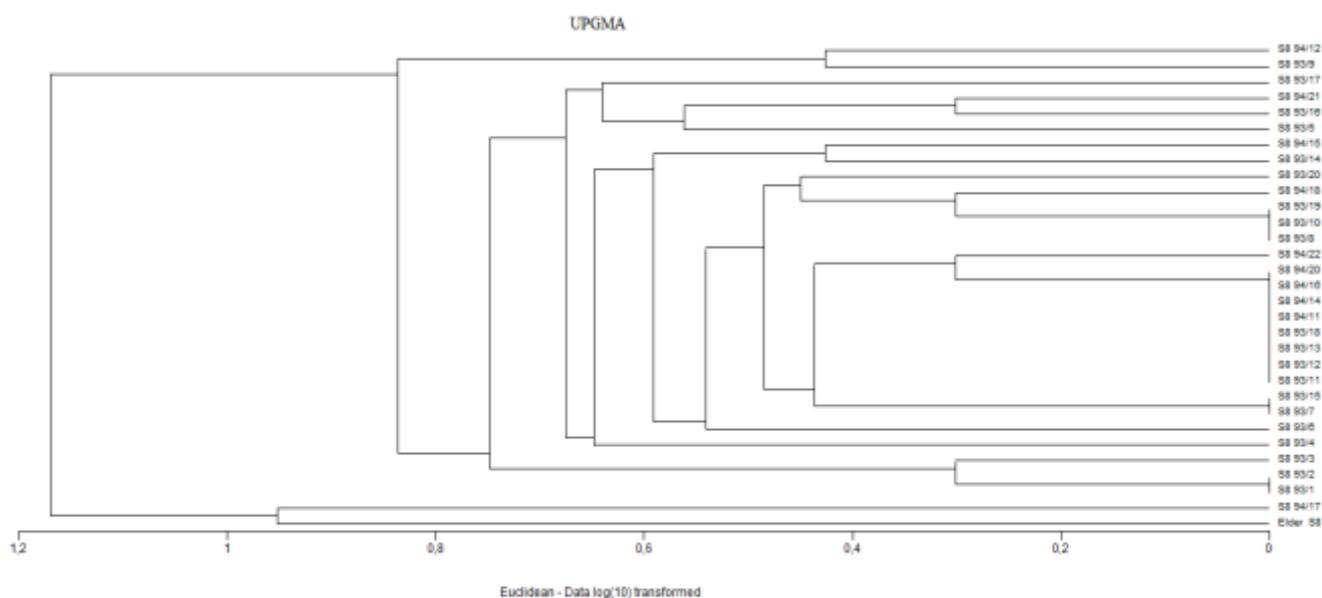


Figure 6. Dendrogram of 31 individual oil palms from Pisifera S8 parents and mature plants from tissue culture propagation based on the length of the polyisoprenoid carbon chain using log transformations (10) with Euclidean square spacing.

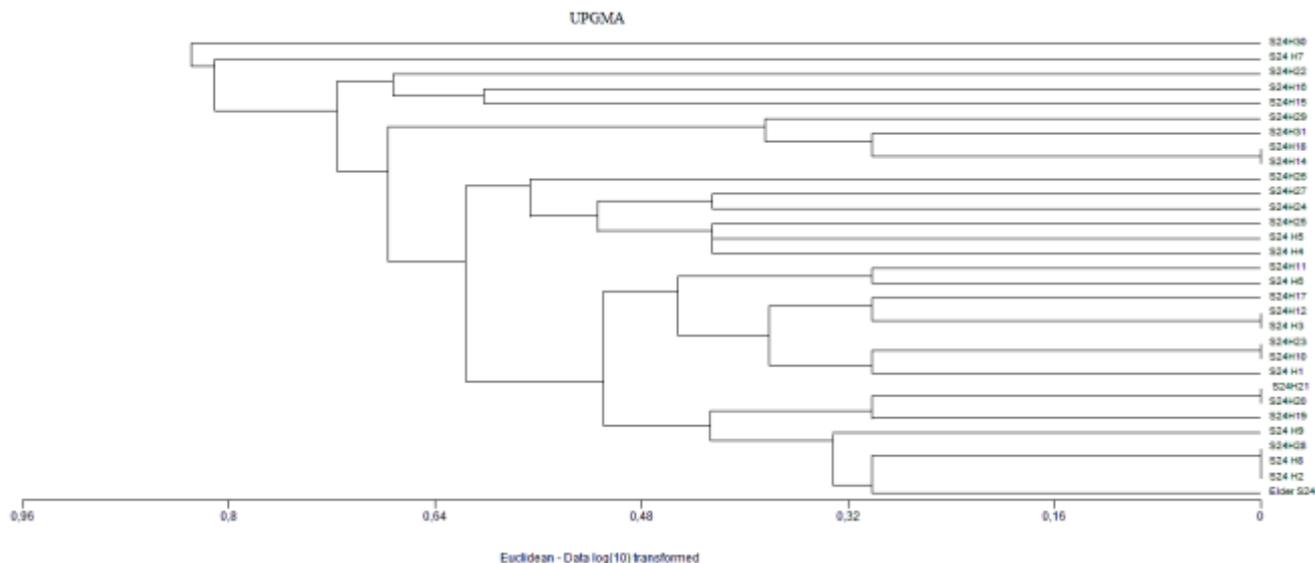


Figure 7. Dendrogram of 31 individual oil palms from Pisifera S24 parents and mature plants from tissue culture propagation based on the length of the polyisoprenoid carbon chain using log transformations (10) with Euclidean square spacing

Several studies have been reported that the profile and occurrence of polyisoprenoids can be used as chemotaxonomic markers. Polyisoprenoids are primarily associated with photosynthetic tissues (Brasher et al. 2015; Basyuni et al. 2016, 2017a,b, 2018a,b). Polyisoprenoid in oil palm leaf tissue occurs in length chain of C45-C65 and C90-C105, while dolichol occurs in C85-C105 (Arifiyanto 2017; Basyuni et al. 2018b). Although studies related to the biological activity of polyisoprenoid and dolichol are still limited, in general, the results of the study explain that these two secondary metabolite compounds are involved in plant defense mechanisms against abiotic and biotic stress (Basyuni et al. 2017a; Baczewska et al. 2014). Afandi et al (2019) reported that there was a different expression of polyisoprenoid profiles concerning the level of plant resistance to *Ganoderma boninense* attack. This difference in expression is also related to its presence in different tissues, treatments, and tolerance levels of plants. The study also explained that the polyisoprenoid profile and polyisoprenoid carbon chain patterns in the root tissue of healthy oil palms could be distinguished from those infected with *G. boninense*. High polyisoprenoid content (2.5 times) has been also reported on tobacco plant leaves inoculated with tobacco mosaic virus (TMV) (Bajda et al. 2009). This result reinforces the idea of polyisoprenoid involvement in plant resistance to pathogens.

Cluster analysis of individuals propagated by tissue culture according to their respective parents

The result of TLC analysis, i.e., the carbon-chain composition from 30 number individuals from in vitro propagation with Pisifera parent S8 and S24 was visualized by dendrogram using the UPGMA method. The dendrogram classifies plants into the cluster of desired traits based on the similarity of their carbon chain

composition. Figure 6 showed 31 oil palm individuals propagated by tissue culture from parent S8 were grouped into two main groups based on the carbon chain of leaves tissue. The first group consists of Elder S8 and S8 94/17, linked because they both have the C80 and C85 chains in the polyisoprenoid, and both lack the C80 and C85 chains in the dolichol compound. The second group consists of 29 individuals outside of the first group. However, based on the euclidean distance of 0.8, the oil palm leaves were grouped into three groups: the I sub-group consisted of Elder S8 and S8 94/17. Sub-group IIA consisted of S8 94/12 and S8 93/9 associated with the presence of C50, C55, and C60 chains in polyisoprenoid compounds, the presence of C50 and C55 chains in their dolichol compounds, and the two individuals did not have C80-C110 chains in dolichol. The IIB sub-group consisted of S8 93/1, S8 93/2, S8 93/3, S8 93/4, S8 93/5, S8 93/6, S8 93/7, S8 93/8, S8 93/10, S8 93/11, S8 93/12, S8 93/13, S8 93/14, S8 93/15, S8 93/16, S8 93/17, S8 93/18, S8 93/19, S8 93/20, S8 94/11, S8 94/14, S8 94/15, S8 94/16, S8 94/18, S8 94/20, S8 94/21, and S8 94/22. Sub-group IIB is classified in one cluster due to the presence of C45 and C65 chains in polyisoprenoid compounds in each individual.

Figure 7 showed 31 oil palm individuals propagated by tissue culture from parent S24 were grouped into three main groups based on the carbon chain of leaves tissue based on the euclidean distance of 0.8. Group I consists only of S24 H30 because only this individual has the C100 chain on the polyisoprenoid. Group II also consisted of only one individual, namely S24 H7, because only this individual had the C45 chain but did not have the C80-C105 chain on the dolichol. Whereas Group III consisted of S24 parents along with 28 other individuals who were propagated by tissue culture from S28 parents, namely S24 H1, S24 H2, S24 H3, S24 H4, S24 H5, S24 H6, S24 H1,

S24 H8, S24 H9, S24 H10, S24 H11, S24 H12, S24 H14, S24 H15, S24 H16, S24 H17, S24 H18, S24 H19, S24 H20, S24 H21, S24 H22, S24 H23, S24 H24, S24 H25, S24 H26, S24 H27, S24 H28, S24 H29, S24 H30, and S24 H31. Group III was grouped into one cluster with respect to the presence of C50, C5, and C60 in dolichol, except as stated from the characteristics of groups I and II.

Carbon chain lengths of dolichol and polyprenol vary from tissue to tissue within the same species and form the predominance of different families concerning growing, treating, or stressing the abiotic and biotic environments. Tateyama et al. (1999) that the distribution of long-chain polyprenol was not always the same as long-chain dolichol in the same organ. Oil palm plants that grow in different areas may differ in the length of the carbon chains of polyisoprenoid compounds (Basyuni et al. 2018b).

From the description above, it can be concluded that the oil palm leaves from in vitro propagation showed more diversity in the number and type of carbon chain lengths in the polyprenol and dolichol compounds compared to the Pisifera S8 and S24 parents. Even though the plants produced by in vitro culture have long-chain types of carbon polyprenol and dolichol which are also owned by the Pisifera S8 and S24 parents as the source of the explants. The diversity that occurs in the type and number of carbon chains that make up polyprenol and dolichol compounds in vitro propagated plants may occur due to somaclonal variations in vitro morphogenesis/embryogenesis process or the effect of environmental factors such as abiotic or biotic stress that occurs during growth and crop development in the field.

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