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Full Length Article

Detection of Polyisoprenoid Composition in Quick Starter and Slow Starter Clones of *Hevea brasiliensis*

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Abstract

The rubber plant (*Hevea brasiliensis* Mull. Arg.) is the only commercial source of natural rubber. The two-plate thin-layer chromatography (2P-TLC) method was used to analyse polyisoprenoid alcohols consisting of dolichols and polyprenols from the leaves of the rubber plant *H. brasiliensis* as clone entres. There were 33 clones based on the metabolism of latex and whether they were quick starters, slow starters, or unidentified. The distribution of polyisoprenoids in *H. brasiliensis* clones were mostly detected as type II in the presence of both polyprenols and dolichol with 31 clones from 33 clone entres. Two clones, IRR 5 and PB 217, were classified as type III with a majority of polyprenols as compared to dolichols. Furthermore, type I of polyisoprenoidswith a dominance of dolichols over polyprenols were not detected. Additionally, it was found that the total lipid content range was largest in IRR 220 and the smallest in RRIM 712. Furthermore, the number of polyisoprenoids was the highest in RRIM 921 and the lowest in PB 217. Moreover, polyprenols and dolichols were abundantly detected in *H. brasiliensis* leaves. Larger amounts of polyprenols (C45-C80), as compared to other clones, were found in IRCA 804. Likewise, the highest content of dolichols (C75-C115) was observed in GT 1. However, the dendrogram analysis did not differentiate between quick starter and slow starter clones, suggesting the similarities of clones based on the data on polyisoprenoids of carbon chain length. © 2020 Friends Science Publishers

Keywords: Dolichol; *Hevea brasiliensis*; Polyprenol; Polyisoprenoids

Introduction

Natural rubber is produced from more than 300 genera and 2500 plant species. Among the species of natural rubber producing plants, the rubber tree (*Hevea brasiliensis* Mull. Arg.) is the only commercial source at this time because of its high rubber content and quality (Venkatachalam *et al.* 2013). As such, rubber biosynthesis in the molecular mechanism of most of these plants needs further investigation. Accordingly, intensive research has been carried out to find and characterize key enzymes and specific compounds involved in rubber biosynthesis in *H. brasiliensis*, *Ficus elastica*, and guayule (*Parthenium argentatum*), which have been reported (Mooibroek and Cornish 2000).

The development of rubber trees breeding research has produced many clones with different characteristics. Rubber trees clustering based on time of peak production resulted in the creation of two groups known as quick starter (QS) and slow starter (SS) clones (Woelan *et al.* 2012). Quick starter clones have peak production in the early tapping period, whereas slow starter clones have peak production in the mid-economic cycle (Woelan *et al.* 2012). Nevertheless, each clone has a specific physiological character based on the response of plants to the applied tapping system that also varied (Obouayeba *et al.* 2010). Moreover, clones with high metabolism tend to be responsive to high intercepts but less responsive to stimulants. Clones with moderate and low metabolism tend to be responsive to stimulant administration but require a longer intercepting interval. Therefore, a secondary biosynthesis of metabolism is needed the specific compounds responsible for the production and tapping of the two clones (Steinbüchel 2003).

A number of studies have been conducted on the potential and actual applications of isoprenoids that comprise a very diverse group of metabolites in the chemical industry, as well as in the fields of medicine and agriculture (Swiezewska and Danikiewicz 2005; Lipko and Swiezewska 2016). Such actions are generally improved if the initial biosynthetic pathway can be recognized, the regulatory mechanisms committed for biosynthesis of

isoprenoids may also be identified (Lipko and Swiezewska 2016). Moreover, two types of polyisoprenoids have been reported in terms of the OH-terminal (α-) isoprene units (Swiezewska *et al*. 1994; Basyuni *et al*. 2016). These polyisoprenoids include dolichol (α-saturated) and polyprenol (α-unsaturated) compounds. The composition of polyisoprenoids has been reported in tropical and subtropical plants (Swiezewska *et al*. 1994; Basyuni *et al*. 2016, 2017) including rubber plants (Tateyama *et al*. 1999) and other organisms such as animals (Sagami *et al*. 1992; Rezanska and Votruba 2001; Ishiguro *et al*. 2014), bacteria (Wolucka *et al*. 1994), fungi (Wojtas *et al*. 2004), andyeast (Grabinska and Palamarczyk 2002).

This study focused on developing a two-plate thin-layer chromatography (2P-TLC) method that effectively and efficiently separates the mixtures of dolichols and polyprenols (Sagami *et al.* 1992; Basyuni *et al.* 2016, 2017). Thus, it aims to analyse the composition of polyisoprenoid from the clones of *H. brasiliensis* leaves entres derived from PT Socfin Indonesia collections based on the metabolism of latex whether they are quick starters, slow starters, or unidentified.

Materials and Methods

Chemicals

The standards of polyprenol (C55-C60) and dolichol (C55-C65) were used to identify the pattern of polyisoprenoids in this study (Basyuni *et al.* 2016, 2017). The silica gel 60 TLC plates, reverse-phase silica RP-18HPTLC plates, other solvents, and chemicals used were of reagent grade (Merck, Darmstadt, Germany).

Plant materials

Samples of rubber tree leaves of 33 clones entres were collected in triplicate from nurseries of PT Socfin Indonesia, Tanah Besih, Tebing Syahbandar, Serdang Bedagai Regency, North Sumatra Province, Indonesia. These sampling sites were located at 3˚19.579' north latitudes and 99˚12.971' east longitudes. The samples consisted of IRCA 18, IRCA 19, IRCA 101, IRCA 130, IRCA 230, IRCA 317, IRR 5, IRR 220, IRR 221, PB 260, PB 340, and RRIM 901 identified as quick starter clones. On the other hand, BPM 1, BPM 24, GT 1, IRCA 41, PB 217, PR 300, RRIC 100, RRIM 600, RRIM 712, and RRIM 921 were recognized as slow starter clones. Some of the clones were not classified yet and include IRCA 331, IRCA 427, IRCA 804, RRIM 908, RRIM 911, RRIM 2020, IRR 118, IRR 429, IRR 440, PC 10, and PM 10.

Procedures

Polyisoprenoid isolation: This study followed the procedure for polyisoprenoid isolation as described by Sagami *et al.* (1992): leaves were dried at 60–75°C for 48 h. The leaf tissue (2 g each) were then homogenised to a fine powder and macerated in 30 mL of a chloroform/methanol (2: 1 v/v) solvent for two days. Lipid extracts from leaves were saponified at 65ºC for 24 h in concentrations of 0.45 g KOH, 2 mL of ethanol, and 2 mL of distilled water. Subsequently, the NSL (non saponifiable lipids) of each tissue were dissolved with hexane solvent and ready to be analysed.

Two-plate thin layer chromatography (2T-TLC) analysis: The first-plate of TLC was performed for 60 min on silica gel (20 × 3 cm) with a toluene-ethyl acetate (9: 1) solvent system as explained earlier (Basyuni *et al*. 2016, 2017). The longitudinal edge of the first dimension of TLC had a width of 1 cm and the zone of concentration of the reverse phase RP-18 TLC was clamped using two magnetic bars (4.0 × 1.1 × 0.8 cm) facing each gel phase.The bound TLC plate was then developed perpendicular to the first plate for transfer of dolichol and polyprenol to the concentration zone in the reverse TLC phase.The second-plate reverse phase of the RP-18 silica gel TLC was carried out with acetone solvent for about 30 min. The position of the polyisoprenoid alcohol was differentiated and developed through a two-plate TLC silica gel and then determined and visualized with iodine vapor. Chromatographic images were acquired and scanned by Cannon E-400 series printer. Polyprenols and dolichols were traced on RP-18 HPTLC plates using Image J v. 1.46r (Schneider *et al*. 2012) with polyprenol and dolichol standards as a reference. The scan chromatogram was in grayscale mode and was evaluated for plot lines and peak labels. The highlight of this area was copied and pasted into the Microsoft Excel program.

Data analysis

Dendograms were drawn based on cluster analysis and included polyprenols and dolichols from 33 clone entries. In this study, all data were log (10) transformed. Subsequently, a cluster analysis was used to draw a dendrogram that represents leaf data using the unweighted-pair group method with arithmetic mean (UPGMA) using MVSP (multivariate statistical package) v. 3.22 (Kovach Computing Service). The criteria for cluster combinations were determined by the Euclidean distance.

Results

Occurrence and profile of polyisoprenoids in rubber plants

Table 1 shows the quantitative analysis of polyprenol and dolichols content in 33 clone entres of rubber tree leaves. The total lipids were measured gravimetrically and estimated as a fraction of crude lipids. The TL content was largest in IRR 220 (1.09 mg g^{-1} dry weight) and the smallest in RRIM 712 (0.47 ± 0.40 mg g^{-1} dry weight). The quantity

Table 1: Distribution of polyprenols and dolichols in rubber plants

Clones	TL	PI	Pol	Dol	% in total lipid			% in PI		Type
	(mg/g dw)	(mg/g dw)	(mg/g)	(mg/g)	PI	Pol	Dol	Pol	Dol	
IRCA 18	0.72 ± 0.01	5.49	3.29	2.20	762.42	456.69	305.72	59.90	40.10	II
IRCA 19	0.73 ± 0.03	3.89	2.19	1.70	533.04	299.98	233.07	56.28	43.72	II
IRCA 41	0.70 ± 0.02	2.69	2.00	0.69	384.75	285.57	99.18	74.22	25.78	II
IRCA 101	0.73 ± 0.04	4.73	3.13	1.59	647.72	429.26	218.47	66.27	33.73	II
IRCA 130	0.77 ± 0.07	5.37	3.40	1.96	697.04	442.03	255.01	63.42	36.58	II
IRCA 230	0.79 ± 0.06	2.36	1.33	1.03	299.01	168.61	130.40	56.39	43.61	II
IRCA 317	0.78 ± 0.03	4.94	2.83	2.11	632.96	362.32	270.64	57.24	42.76	II
IRCA 331	0.68 ± 0.01	2.26	1.03	1.23	331.95	151.06	180.89	45.51	54.49	II
IRCA 427	1.02 ± 0.02	7.38	5.56	1.82	723.07	544.74	178.33	75.34	24.66	II
IRCA 804	0.87 ± 0.04	6.33	4.70	1.63	727.21	539.77	187.44	74.22	25.78	II
RRIM 600	0.77 ± 0.02	2.61	1.70	0.91	338.84	220.79	118.05	65.16	34.84	II
RRIM 712	0.47 ± 0.40	2.60	1.68	0.92	552.43	356.47	195.96	64.53	35.47	II
RRIM 901	0.65 ± 0.14	5.15	3.92	1.23	792.89	603.62	189.27	76.13	23.87	II
RRIM 908	0.65 ± 0.43	3.76	1.66	2.10	578.18	255.81	322.36	44.25	55.75	II
RRIM 911	0.81 ± 0.13	4.35	2.36	2.00	537.17	290.81	246.36	54.14	45.86	II
RRIM 921	0.79 ± 0.04	8.89	6.17	2.71	1124.99	781.45	343.54	69.46	30.54	II
RRIM 2020	0.74 ± 0.09	5.31	3.09	2.22	747.80	417.04	300.44	58.13	41.87	II
IRR 5	0.66 ± 0.20	4.63	4.30	0.33	712.87	662.25	50.63	92.90	7.10	III
IRR 118	0.72 ± 0.07	6.31	3.44	2.87	876.51	478.05	398.46	54.54	45.46	II
IRR 220	1.09 ± 0.58	5.49	3.42	2.08	504.12	313.68	190.43	62.22	37.78	II
IRR 221	0.74 ± 0.05	4.21	2.05	2.16	569.46	277.00	292.46	48.64	51.36	II
IRR 429	0.48 ± 0.41	4.16	2.75	1.41	867.25	572.68	294.57	66.03	33.97	II
IRR 440	0.71 ± 0.02	5.20	3.58	1.62	732.25	504.55	227.70	68.90	31.10	II
PB 217	0.65 ± 0.22	2.02	2.02	0.00	310.61	310.61	Nd	100.00	Nd	III
PB 260	0.69 ± 0.03	1.74	1.18	0.56	252.63	170.92	81.71	67.66	32.34	II
PB 340	0.70 ± 0.07	4.22	2.53	1.70	603.31	360.87	242.45	59.81	40.19	II
BPM 1	0.70 ± 0.01	3.84	2.10	1.74	548.86	299.90	248.96	54.64	45.36	II
BPM 24	0.75 ± 0.02	3.20	2.04	1.16	426.88	271.84	155.05	63.68	36.32	II
GT 1	0.71 ± 0.005	4.31	1.85	2.46	607.40	261.02	346.38	42.97	57.03	II
PC 10	0.78 ± 0.02	3.29	1.89	1.40	421.28	242.06	179.23	57.46	42.54	II
PM 10	0.80 ± 0.05	2.89	1.83	1.06	360.99	228.18	13281	63.21	36.79	II
PR 300	0.85 ± 0.14	2.51	1.47	1.04	295.30	172.45	16065	58.40	41.60	II
RRIC 100	0.80 ± 0.03	7.19	5.04	2.15	898.78	629.54	26924	70.04	29.96	II

Nd= not detected, TL= Total lipids, PI= Polyisoprenoids, Pol= Polyprenols, Dol= Dolichols. Data are the mean of triplicate analyses
TL was presented as means of triplicate analyses ± SD

of polyisoprenoids was the highest in RRIM 921 (8.89 mg g^{-1}dry weight) and lowest in PB 217 (2.02 mg g^{-1} dry weight). Both RRIM 921 and PB 217 were slow starter clones.

Table 2 shows the profiles and occurrence of polyprenols and dolichols along with the length of the carbon chains for each type. Moreover, the separation of polyisoprenoids into polyprenol and dolichol were performed by 2P-TLC. It is worth noting that PB 217 is prominent from the others in that clone entres consist of shorter-chain polyprenols only namely ficaprenol-like chain length (C60-C65). Meanwhile GT 1, a member of SS clone, was noticeable with ficaprenol chain length (C50-C60) and a longer chain length of dolichol (C75-C115). However, among the clones, only IRCA 331 and IRR 221 had longer chain lengths of dolichol (Table 2).

2P-TLC chromatograms analysis

Fig. 1–6 show 2P-TLC chromatograms hexane extracts of polyisoprenoids from 33 clones. There was similarity in the polyprenol carbon-chain lengths (C40-C65) in IRCA 101 (Fig. 1d), IRCA 130 (Fig. 1e), RRIM 901 (Fig. 3c) and IRR 220 (Fig. 4b). Four of these clones were categorised as quick starter. Furthermore IRCA 18 (Fig. 1a), RRIM 600 (Fig. 2e), RRIM 911 (Fig. 3c), IRR 429 (Fig. 4d), PB 340 (Fig. 5b), PM 10 (Fig. 6a) had a similarity in carbon-chain lengths of polyprenol (C45-C60).

In the case of dolichols, there was similarity in terms of the following carbon chain lengths among the clones: C65-C85, C70-C80, C70-C85, and C75-C85. Dolichols with carbon chain length of C65-C85 consisted of IRCA 804 (Fig. 2d) and BPM 1 (Fig. 5c). Dolichols with carbon chain length of C70-C80 comprised three clones: IRCA 10 (Fig. 1b), RRIM 600 (Fig. 2e), PM 10 (Fig. 6a). Meanwhile, dolichols with the carbon chain length of C70-C85 consisted of seven clones: IRCA 101 (Fig. 1d), IRCA 427 (Fig. 2c), RRIM 901 (Fig. 3a), RRIM 2020 (Fig. 3e), IRR 220 (Fig. b), IRR 440 (Fig. 4e), and PR 300 (Fig. 6b). Dolichols with carbon chain length of C75-C90 were as follows: IRCA 230 (Fig. 1f), RRIM 908 (Fig. 3b), RRIM 921 (Fig. 3d), IRR 5 (Fig. 3f), IRR 118 (Fig. 4a), and PB 260 (Fig. 5a).

Cluster analysis of polyisoprenoids data

The carbon-chain length pattern was analysed and translated into binary data and visualized into a dendrogram using the UPGMA method. The cluster analysis was separated into

Table 2: Polyprenol and dolichol carbon-chain lengths distributing in 33 clones of rubber plants

Clones	C43	Polyprenol	Dolichol
IRCA 18	O	45 50 55 60	65 70 75 80
IRCA 19	O	40 45 50 55	70 75 80
IRCA 41	O	50 55 60	75 80 85
IRCA 101	O	40 45 50 55 60 65	70 75 80 85
IRCA 130	O	40 45 50 55 60 65	70 75 80 85 90
IRCA 230	O	50 55 60	75 80 85 90
IRCA 317	O	45 50 55 60 65 70	60 65 70 75 80 85 90 95
IRCA 331	O	45 50 55	70 75 80 85 90 95 100
IRCA 427	O	40 45 50 55 60	70 75 80 85
IRCA 804	O	45 50 55 60 65 70 75 80	65 70 75 80 85
RRIM 600	O	45 50 55 60	70 75 80
RRIM 712	O	50 55 60	80 85 90
RRIM 901	O	40 45 50 55 60 65	70 75 80 85
RRIM 908	O	45 50 55 60 65 70	75 80 85 90
RRIM 911	O	45 50 55 60	70 75 80 85 90 95
RRIM 921	O	45 50 55 60 65	75 80 85 90
RRIM 2020	O	45 50 55	70 75 80 85
IRR 5	O	45 50 55 60 65	75 80 85 90
IRR 118	O	45 50 55	75 80 85 90
IRR 220	O	40 45 50 55 60 65	70 75 80 85
IRR 221	O	40 45 50 55	65 70 75 80 85 90 95 100
IRR 429	O	45 50 55 60	65 70 75 80
IRR 440	O	40 45 50 55	70 75 80 85
PB 217	O	60 65	
PB 260	O	50 55 60	75 80 85 90
PB 340	O	45 50 55 60	50 55 60 65 70 75 80 85
BPM 1	O	50 55 60	65 70 75 80 85
BPM 24	O	50 55 60 65 70	85 90
GT 1	O	50 55 60	75 80 85 90 95 100 105 110 115
PC 10	O	50 55	75 80 85
PM 10	O	45 50 55 60	70 75 80
PR 300	O	45 50 55	70 75 80 85
RRIC 100	O	50 55 60 65	75 80 85

Note: C43 = Bombiprenone. O = detected

two groups based on the node. Group 1 included IRCA 18, IRCA 101, IRCA 230, RRIM 600, RRIM 921, RRIM 2020, IRCA 19, IRCA 41, RRIM 908, IRCA 331, and IRCA 317. Group 2 included IRR 429, RRIM 901, IRR 220, PB 260, PM 10, IRR 5, PR 300, IRR 440, IRCA 130, IRCA 427, RRIM 712, RRIC 100, RRIM 911, IRR 118, BPM 1, IRR 221, BPM 24, IRCA 804, PB 340, PB 217, and GT 1. Moreover, IRR 220, RRIM 901, IRCA 101, and IRCA 130 were close to each other as quick starter clones (Fig. 7).

Discussion

The grouping of rubber clones based on latex metabolism becomes important as a reference for planters for the choice of planting material to be used on a commercial scale. Furthermore, it is also important in determining the expected composition of latex production in each field so that it can be sustainable. In this study, the grouping was based on polyisoprenoid composition. More specifically, the polyisoprenoids composition in rubber tree leaves in Indonesia with typological clone quick starters and slow starters indicated the presence of both polyprenols and dolichols, with polyprenols being slightly higher in content than dolichols. In *H. brasiliensis* leaves, polyprenols were abundantly detected, especially in IRR 5 as quick starter and PB 217 as slow starter clones. This finding agreed with the findings of an earlier study on polyprenols' predominance over dolichols in plant leaves (Tateyama *et al.* 1999; Swiezewska and Danikiewicz 2005). On the other hand, shorter chains of polyprenols has been found in young and old rubber leaves (Tateyama *et al.* 1999), soybean leaves (Kurisaki *et al.* 1997), and immature spinach leaves (Sakaihara *et al.* 2000) in the families of Lauraceae, Magnoliaceae, and Tiliaceae (Roslinska *et al.* 2002) as well as immature mangrove leaves (Basyuni *et al.* 2016, 2017; Basyuni and Wati 2018).

In this study, most clones (93.9%) were detected as type II in the presence of mutually polyprenol and dolichol with 31 clones from 33 clones entres. Two clones, IRR 5 and PB 217 were classified as type III, with there being a majority of polyprenols over dolichols. Furthermore, typer I of polyisoprenoids had a dominance of dolichol over polyprenol, which was not detected. In contrast to this present study that consisted of type II and III, it has been previously reported, the fundamental types of distribution of polyprenols and dolichols in plant tissue has been grouped into three types: I, II, and III (Basyuni *et al.* 2016, 2017). In the NCBI database, a previous study described four probable polyprenol reductase genes from *H. brasiliensis* (Basyuni *et al.* 2019). Polyprenol reductase as an enzyme converted polyprenol to dolichols in the biosynthesis of dolichols of mangrove plants (Basyuni and Wati 2018). Two clones, IRR 5 and PB 217, were categorized as type III with a majority of polyprenols over dolichols.

In this context, Swiezewska and Danikiewicz (2005) proposed the localisation of ficaprenols in chloroplast. The ficaprenols were found in this study and other plants (Kurisaki *et al.* 1997; Sakaihara *et al.* 2000). The occurrence of phytol may be in the biosynthesized ficaprenols in this organelle (Sakaihara *et al.* 2000). The dolichols were not detected in PB 217, where the presence of polyprenols was at 100%.

The terminology of squick starter and slow starter clones based on the genotype differences in latex metabolism,includes inorganic phosphorus and high sucrose contents. According to the study with two clones, different concentrations of sucrose in latex determine the grouping of clones into two types as in clones PB 260 and PR 300. PB 260 is a quick starter clone with high metabolism while PR 300 is a slow starter clone with low metabolism (Martiansyah *et al*. 2018). Moreover, PB 260 in the first tapping produces high latex production and directly converts sucrose without stimulants (Herlinawati and Kuswanhadi 2013). Furthermore, it had shorter polyprenol (C50-C60) and dolichol with a carbon chain length of C75-C90.

According to another study by Lacote *et al.* (2010), ethylene stimulation affects the rubber tree in producing more latex, which is correlated to the content of sucrose and inorganic phosphorus in latex cells. IRCA 130 has a quick starter clone with high inorganic phosphorus content and low sugar concentration; in this study, with a carbon chain

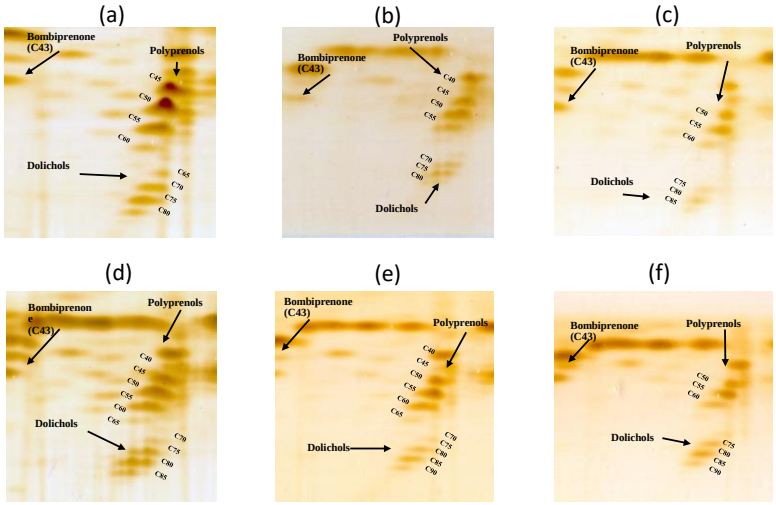

Fig. 1: 2P-TLC chromatograms hexane extracts of polyisoprenoids from (**a**) IRCA 18, (**b**) IRCA 19, (**c**) IRCA 41, (**d**) IRCA 101, (**e**) IRCA 130 and (**f**) IRCA 230. The number indicates the carbon-chain length of the polyiso

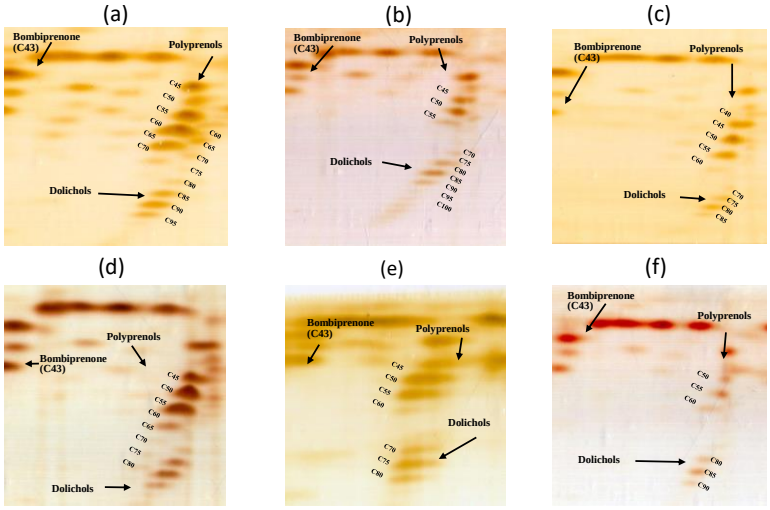

Fig. 2: 2P-TLC chromatograms hexane extracts of polyisoprenoids from (**a**) IRCA 317, (**b**) IRCA 331, (**c**) IRCA 427, (**d**) IRCA 804, (**e**) RRIM 600, and (**f**) RRIM 712

length of polyprenol (C40-C65) and dolichol with a chain lenth of C75-C90, ethylene stimulation was not needed to improve production of latex. For IRCA 230, with more sugar levels, eight times the application of the ethylene stimulant per year is sufficient for the highest yield. However, in slow starter clones with high sucrose levels and low inorganic phosphorus levels, such as PB 217, the increase in latex yield was significant due to the stimulating effect of ethylene. This slow starter clone requires more stimulation to produce more latex with negative impact in the long term. Thus, large scale state or private plantations use ethylene stimulants (2-chloroethylphosphonic acid) to increase latex production. Cumulatively the production of latex and biochemistry of latex cells is strongly influenced by ethylene stimulants (Lacote *et al*. 2010). GT 1, a slow starter clone, was distinguishable from the other clones, that contained longer dolichol (C75-C115). GT 1 has moderate levels of inorganic phosphorus and sucrose and the stimulant application does not have a negative effect. On the other hand, PB 217 with moderate inorganic phosphorus content and high sucrose content responds to high latex production with intensive stimulation (Gohet *et al*. 2003). Meanwhile, IRCA 130 has similarities with IRCA 230 but is different from GT 1 and PB 217 (Serres *et al*. 1988; Gohet *et al*. 2003). More specifically, IRCA 130 and IRCA 230 have a fast metabolism with high inorganic phosphorus concentration and low sucrose content. With such conditions, etephon stimulation in IRCA 130 clones is not necessary because there is no difference between the application and non-application of stimulants. Additionally, the same study was also carried out by Serres et al. (1988) in PB 235 and it was found that PB 260 clones also have high metabolism. High cellular activity in the form of high inorganic phosphorus and PiP0 is an indicator of the

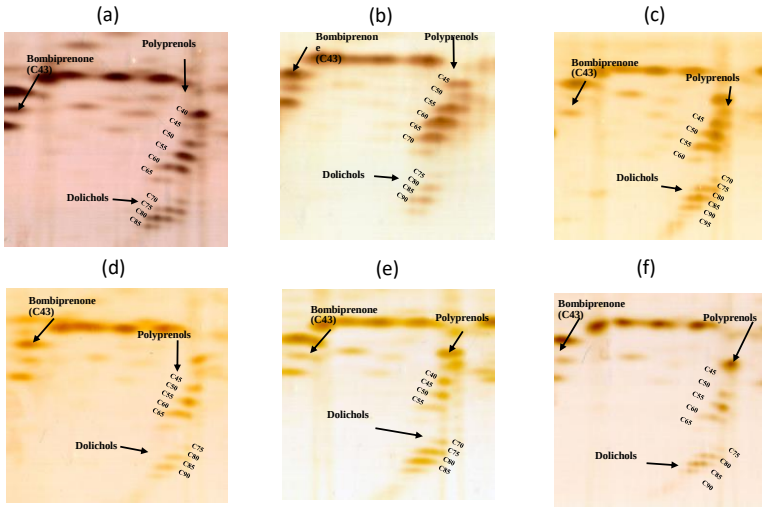

Fig. 3: 2P-TLC chromatograms hexane extracts of polyisoprenoids from (**a**) RRIM 901, (**b**) RRIM 908, (**c**) RRIM 911, (**d**) RRIM 921, (**e**) RRIM 2020, and (**f**) IRR 5

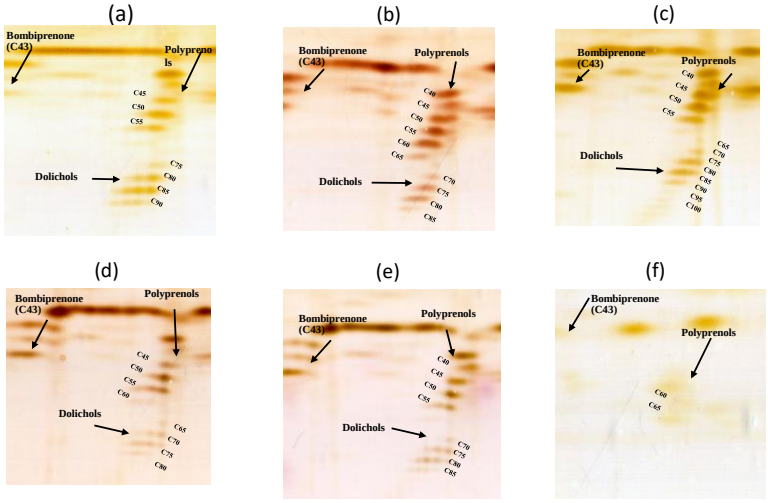

Fig. 4: 2P-TLC chromatograms hexane extracts of polyisoprenoids from (**a**) IRR 118, (**b**) IRR 220, (**c**) IRR 221, (**d**) IRR 429, (**e**) IRR 440, and (**f**) PB 217

presence of highyield clones (including IRCA 130) capable of producing strong latex without stimulants (Jacob et al. 1989).

According to another study, the slow starter clones are GT 1, RRIC 100 and BPM 24 observed as clones resistant against a disease that caused abnormal leaf fall. Moreover, the reduced number of trees produced due to disease causes a significant reduction in the amount of latex produced. The resistance of GT 1 to Corynespora cassiicola in vitro (through a separate leaf technique), in immature leaf tissue with virulent pathogenic strains was tested. It was proven that GT1 is resistant based on the size of the lesion (Roy *et al*. 2019).

Furthermore, it was revealed that the structural portion of phenolic aldehyde lignin produced around infected areas is very significant in the disease resistance of plants. Research on this has been carried out on resistant and susceptible clones (in connection with Phytophthora)in terms of the importance of anti-fungal phenolic compounds to inhibit diverse fungal pathogens (Elmer *et al*. 1994). It was found that high concentrations of phenolic compounds in the petiole of rubber clones were resistant and healthy (BPM 24 and RRIC 100). Furthermore, it is interesting to note that BPM 24 consists of short chain dolichol (C85-C90). Additionally, in infected plants, the enzyme activity in synthesizing phenolic compounds increases. For example, the leaf stalk tissue of resistant clones shows the activity of the enzyme phenylalanine ammonia-lyase (PAL) for catalysis of a precursor synthesis for increased biosynthesis of lignin.

In order to limit the development of pathogens to attack uninfected healthy tissue, plants activate various

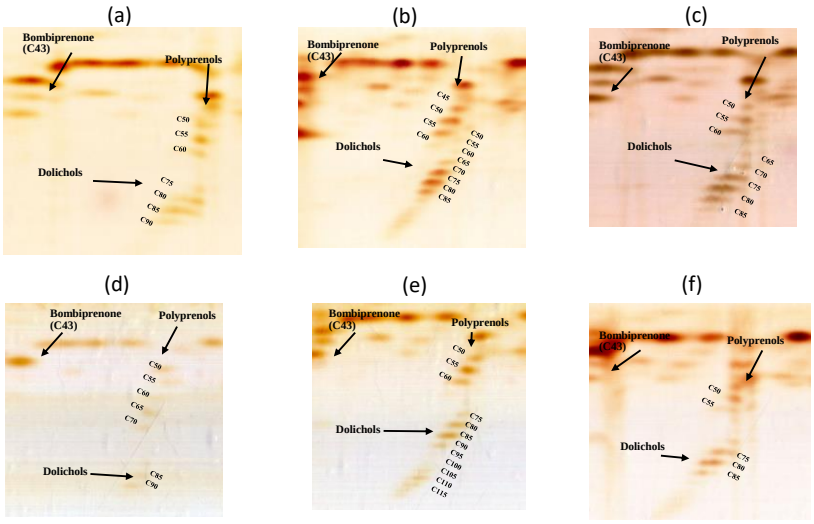

Fig. 5: 2P-TLC chromatograms hexane extracts of polyisoprenoids from (**a**) PB 260, (**b**) PB 340, (**c**) BPM 1, (**d**) BPM 24, (**e**) GT 1, and (**f**) PC 10

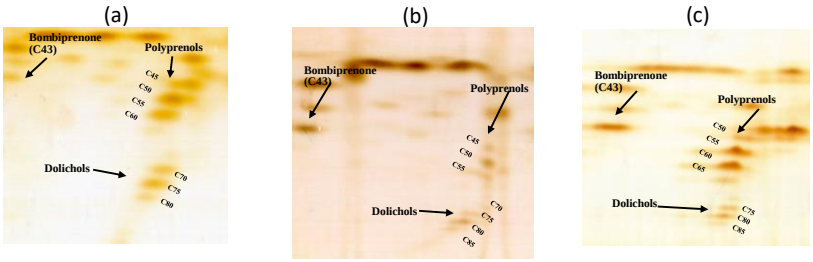

Fig. 6: 2P-TLC chromatograms hexane extracts of polyisoprenoids from (**a**) PM 10, (**b**) PR 300, and (**c**) RRIC 100

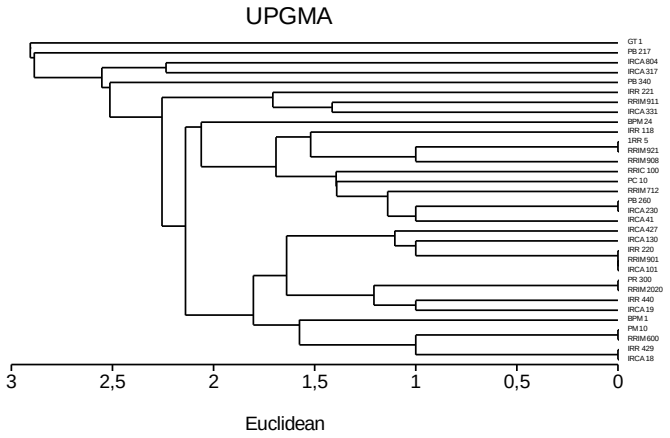

Fig. 7: Dendrogram showing the similarities of species based on carbon-chain length leaves data of polyisoprenoids by log (10) transformation using the Euclidean distance of 33 clones that were quick starter, slow starter, and unidentified; UPGMA: unweighted-pair group method with arithmetic mean

defence mechanisms. Moreover structural changes in cell walls, such as the deposition of lignin, suberin, callus and oxidative bursts, are the initial reaction of plant defences, in the subsequent phase of pathogenesis, expression of pathogenesis-related (PR) genes, antimicrobial proteins, and the production and the causes of phytoalexins accumulation (Wojtaszek 1997).

It has been reported that chromatograms of polyisoprenoids from young and old leaves of rubber plant, shows dolichols with chain lengths of C75-C100 and C65-

C90 were found as the major polyisoprenoid alcohols in young and old leaves, respectively (Tateyama *et al.* 1999). Compared to this study, highercontents of polyprenols (C45-C80) were traced in IRCA 804 as compared to other clones. The largest amounts of dolichols (75–115) were detected in GT 1. Furthermore, traces of heveaprenols (C50-C60) were also detected in IRCA 41, IRCA 230, RRIM 712, PB 260, BPM 1, and GT 1. There are also similarities in the length of carbon chains in GT 1 clones, polyprenol (C50-C60) and dolichols (75–115); in this study, with reference to Tateyama *et al.* (1999), polyprenol (C50-C60) and dolichols (C75-C100) in the studied plant tissue were both found in young leaves.

The dendogram depicts the similarities in leaf polyisoprenoids' carbon-chain length of 33 rubber clone entres of *H. brasiliensis*. The determination of quick starter and slow starter clones was based on the latex metabolism (Siagian and Siregar 2011). The characteristics of quick starter clones have a peak pattern in terms of latex production occurring in the early period, lack responsiveness to stimulants, are prone to tapping panel dryness (TAP) and have thin skin recovery, on the other hand, slow starter clones reach peak production in the mid-tapping period, are responsive to stimulants, are relatively resistant to overexploitation, and have thick skin recovery (Andriyanto and Tistama 2014). Moreover, the cluster does not show the clones entres' relationships. However, it depicts the homologies of clones based on the data on polyisoprenoids. The present study indicated the limitations of the use of a dendrogram; however, molecular data and morphology are also needed to clarify the classification of rubber trees according to metabolism.

In this study, the plant tissue materials were from leaves entres. Entres were the budding source used in rubber grafting. Therefore, in the entres phase, the plants were not tapped to get the rubber latex. Tapping is an activity carried out to obtain sap from rubber plants to which plants respond as a threat to their lives (Nugrahani *et al.* 2016); this is related to the energy of metabolism. Environmental stress will cause the accumulation of reactive oxygen species (ROS) which can destroy the macromolecules that make up organelles or cell membranes. Damage to the membrane will trigger cell death. In order to overcome these stresses, rubber plants increase the activity of ascorbate peroxidase (APX). APX play a role in detoxification of ROS *in vivo*, and in resistance to stress and regulating the duration of latex flow (Tjoet *et al.* 2002). More specifically, polyisoprenoids (natural rubber) as biopolymers from plants function as carbon storage compounds, energy carriers and protective elements against pathogens (Dake 2015) in addition to defending and being a barrier against disturbance from the environment that affects the plant metabolically.

Another study clarified the occurrence of polyisoprenoids in the plantlets of *Periploca sepium* Bunge, although in small traces, and in its milky exudate (Bamba *et al.* 2007). However, due to its low rubber content this plant cannot be used to manufacture rubber on a large scale, but it has proven useful in terms of research on its tissue culture and transformation systems (Miyabashira *et al.* 2003). Moreover, the utlization of *P. sepium* is required to study the mechanism of polyisoprenoid biosynthesis in plant. However, it is suspected that there will be differences and the distribution of polyisoprenoid content in rubber tree plants that have been tapped with plants still in the entres phase, with the main tissue being the milky exudate and the grouping of clones as quick starter and slow starter.

Conclusion

The leaves of *H. brasiliensis* from quick starter and slow starter clones were detected as type II of polyisoprenoids, with the occurrence both polyprenols and dolichol. The cluster analysis of the carbon-chain length pattern was separated into two groups based on the node. The dendrogram did not show a relationship between clone entres; however, the homologies of clones were based on the data on polyisoprenoids of carbon chain length.

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Author Contributions

Collection of *H. brasiliensis* leaves: B.P., L.L., T.C.N., M.B.; Conceptualization: M.B; Investigation: B.P.; Supervision: M.B., T.C.N., L.L.; Draft preparation: B.P.; Writing-review & editing: M.B., B.P. and L.L. All authors read and approved the final manuscript.

References

Andriyanto M, R Tistama (2014). Progress and efforts to overcome tapping panel dryness (TPD) on rubber trees. *Warta Perk* 33:89–102

Bamba T, T Sandoa, A Miyabashira, K Gyokusen, Y Nakazawac, Y Sud, E Fukusakia, A Kobayashia (2007). *Periploca sepium* Bunge as a model plant for rubber biosynthesis study. *Zeits Naturforsch C* 62:579–582

Basyuni M, R Wati (2018). Bioinformatics analysis of the predicted polyprenol reductase gene in higher plants. *J Phys: Conf Ser* 987; Article 012050

Basyuni M, R Hayati, B Pratomo, Lisnawita, H Sagami (2019). Bioinformatics approach of polyprenol reductase in *Hevea brasiliensis*. *J Phys: Conf Ser* 1235; Article 012045

Basyuni M, H Sagami, S Baba, H Oku (2017). Distribution, occurrence, and cluster analysis of new polyprenyl acetones and other polyisoprenoids from North Sumatran mangroves. *Dendrobiology*, 78:18–31

Basyuni M, H Sagami, S Baba, H Iwasaki, H Oku (2016). Diversity of polyisoprenoids in ten Okinawan mangrove. *Dendrobiology* 75:167–175

Dake M (2015). *Biodegradable Polymers: Renewable Nature, Life Cycle, and Applications*, pp:29–56. Microbial Factories Springer New Delhi, India

Elmer WH, MJI Mattina, GJ MacEachern (1994). Sensitivity of plant pathogenic fungi to taxane extracts from ornamental yews. *Phytopathology* 84:1179–1185

Gohet E, P Chantuma, R Lacote, S Obouayeba, K Dian, A Clement-Demange, D Kurnia, JM Eschbach (2003). Latex clonal typology of *Hevea brasiliensis*: physiological modelling of yield potential and clonal response to ethephon stimulation. *Proc Intl Rubb Workshop Exploit Physiol* 203:199–217

Grabinska K, G Palamarczyk (2002). Dolichol biosynthesis in the yeast *Saccharomyces cerevisiae:* An insight into the regulatory role of farnesyl diphosphate synthase. *FEMS Yeast Res* 2:259–265

Herlinawati E, Kuswanhadi (2013). Metabolic activity of several rubber clones at different tapping frequencies and stimulation. *J Rubb Res* 31:110–116

Ishiguro T, Y Morita-Fujimira, Y Shidoji, H Sagami (2014). Dolichol biosynthesis: The occurrence of epoxy dolichol in skipjack tuna liver. *Biochem Biophys Res Commun* 451:277–281

Jacob JL, JC Prevot, D Roussel, R Lacrotte, E Serres, J d'Auzac, JM Eschbach, H Omont (1989). Yield-limiting Factors, Latex Physiological Parameters, Latex Diagnosis, and Clonal Typology. *In: Physiology of Rubb Tree Latex*, pp:345–403. CRC Press, Boca Raton, Florida, USA

Kurisaki A, H Sagami, K Ogura (1997). Distribution of polyprenols and dolichols in soybean plant. *Phytochemistry* 44:45–50

Lacote R, O Gabla, S Obouayeba, JM Eschbach, F Rivano, K Dian, E Gohet (2010). Long-term effect of ethylene stimulation on the yield of rubber trees is linked to latex cell biochemistry. *Field Crops Res*115:94–98

Lipko A, E Swiezewska (2016). Progress in lipid research. Isoprenoid generating systems in plants—A handy toolbox how to assess contribution of the mevalonate and methylerythritol phosphate pathways to the biosynthetic process. *Prog Lipid Res* 63:70–92

Martiansyah I, RA Putranto, N Khumaida (2018). Sucrose analysis in leaf tissue of two contrasted rubber tree clones under abiotic and biotic stresses. *Earth Environ Sci*, 183; Article 012018

Miyabashira A, K Gyokusen, Y Nakazawa, E Fukusaki, T Bamba, A Kobayashi, Y Su (2003). Regeneration from *Periploca sepium* Bunge stem and leaf. *Bull Kyusyu Univ* 84:43–50

Mooibroek H, K Cornish (2000). Alternative sources of natural rubber. *Appl Microbiol Biotechnol* 53:355–365

Nugrahani MO, A Rouf, I Berlian, H Hadi (2016). Physiological study of tapping panel dryness in rubber (*Hevea brasiliensis*). *Warta Perkaret* 35:135–146

Obouayeba S, EF Soumahin, LF Coulibaly (2010). Low intensity tapping systems applied to clone PR 107 of *Hevea brasiliensis* (Muell. Arg.) in South-eastern Côte d'Ivoire: Influence of the nature of the exploited bark and the position of tapping panel. *Agric Biol J North Amer* 1:1106–1118

Rezanska T, J Votruba (2001). Chromatography of long chain alcohols (polyprenols) from animal and plant sources. *J Chromatogr A* 936:95–110

Roslinska M, K Walinska, E Swiezewska, T Chojnacki (2002). Plant long-chain polyprenols as chemotaxonomic markers. *Dendrobiology* 47:41–50

Roy CB, H Liu, A Rajamani, T Saha (2019). Transcriptome profiling reveals genetic basis of disease resistance against *Corynespora cassiicola* in rubber tree (*Hevea brasiliensis*). *Curr Plant Biol* 17:2–16

Sagami H, A Kurisaki, K Ogura, T Chojnacki (1992). Separation of dolichol from dehydrodolichol by a simple two-plate thin layer chromatography. *J Lipid Res* 33:1857–1862

Sakaihara T, A Honda, S Tateyama, H Sagami (2000). Subcellular fractionation of polyprenyl diphosphate synthase activities responsible for the syntheses of polyprenols and dolichols in spinach leaves. *J Biochem* 128:1073–1078

Schneider CA, WS Rasband, KW Eliceiri (2012). NIH image to ImageJ: 25 years of image analysis. *Nat Meth* 9:671–675

Serres E, A Cle´ment, JC Prevot, JL Jacob, J Comme`re, R Lacrotte, JM Eschbach (1988). Clonal typology of laticiferous producing vessels in *Hevea brasiliensis. In: Intl Rubber Res Dev Board Expl Physiol Ame*, pp:231–246. Hevea IRCA-CIRAD Montpellier, France

Siagian N, THS Siregar (2011). Tapping quality checks to support high productivity. *Warta Perkaret* 30:26–33

Steinbüchel A (2003). Production of rubber-like polymers by microorganisms. *Curr Opin Microbiol* 6:261–270

Swiezewska E, W Danikiewicz (2005). Polyisoprenoids: Structure, biosynthesis, and function. *Progr Lipid Res* 44:235–258

Swiezewska E, W Sasak, T Mankoowski, W Jankowski, T Vogtman, I Krajewska, J Hertel, E Skocylas, T Chojnacki (1994). The search for plant polyprenol. *Acta Biochin Pol* 41:235–258

Tateyama S, R Wititsuwannakul, D Wititsuwannakul, S Sagami, K Ogura (1999). Dolichols of rubber plant, ginkgo and pine. *Phytochemistry* 51:11–15

Tjoet VD, JC Prevot, JL Jacob, AV Clement (2002). Both ascorbate peroxidase and glutathione peroxidase are active in removal of H_2O_2 from cytosol of *Hevea brasiliensis* latex. *J Rubber Res* 5:226–243

Woelan S, Junaidi, AP Syarifah (2012). Optimizing yield IRR 200 series using some tapping systems in plot promotion trial. *Ind J Nat Rubber Res* 30:75–85

Wojtas M, T Bieñkowski, S Tateyama, H Sagami, T Chojnacki, W Danikiewicz, E Swiezewska (2004). Polyisoprenoid alcohols from the mushroom *Lentinus edodes*. *Chem Phys Lipids* 130:109–115

Wojtaszek P (1997). Oxidative burst: An early plant response to pathogen infection. *J Biochem* 322:681–692

Wolucka BA, MR McNeil, ED Hoffmann, T Chojnacki, PJ Brennan (1994). Recognition of the lipid intermediate for arabinogalactan/ arabinomannan biosynthesis and its relation to the mode of action of ethambutol on mycobacteria. *J Biol Chem* 269:23328–23335

Venkatachalam P, N Geetha, P Sangeetha, A Thulaseedharan (2013). Natural rubber producing plants: An overview. *Afr J Biotechnol* 12:1297–1310