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Effects of plant growth regulator from seaweed extracts on expression of genes regulating the oil and related metabolites productions in oil palm

IRMA KRESNAWATY[1,2,♥], DJOKO SANTOSO[1], SUMI HUDIYONO[2]

[1]Indonesian Research Institute for Biotechnology and Bioindustry. Jl. Taman Kencana No. 1, Bogor 16128, West Java, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. Jl. Lingkar Kampus Raya, Depok 16424, West Java, Indonesia. Tel.: +62-21-7270027, ♥email: Irma.kresnawaty@yahoo.com

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Abstract. *Kresnawaty I, Santoso D, Hudiyono S. 2022. Effects of plant growth regulator from seaweed extracts on expression of genes regulating the oil and related metabolites productions in oil palm. Biodiversitas 23: 793-800.* Plant growth regulators (PGRs) have been used in agricultural practices to regulate various processes, such as germination, vegetative growth, reproduction, maturation, leaf senescence and post-harvest. Seaweed extract as natural PGRs source contains auxin, cytokines, and gibberellins. Although PGRs have been used for decades, the effects of these compounds on the production of secondary metabolites, proteins and related gene-expression are not yet well known in some perennial crops. To study those, treatments consist of trunk injection of oil palm trees with biostimulant that contain PGRs from seaweed extract. Effects of treatments compared were biostimulant (B), biostimulant with activator for oil accumulation (BA) and control (C). The results indicated that the biostimulant treatments affected ACCase Biotin Carboxylase and HMG-CoA genes expression ratio and were maximum at 4 weeks after treatments (WAT) for biostimulant with activators treatments and 8 WAT for biostimulants without activator. Effect of biostimulants treatments in inducing the gene expression was not significant after 8 WAT. Moreover samples with addition biostimulant in 1 and 7 had the highest values of the gibberellin (GID) gene expression. Oil accumulation showed increase in 4 WAT dan had optimum level in 8 WAT because of activators addition, especially increased of palmitic and linoleic acid.

Keywords: ACCase, gene expression, biostimulants, seaweed extracts, fatty acids

INTRODUCTION

Metabolite biosynthesis in plants does not only depend on genetic factors and stages of plant development, but also on plant growth regulators (PGR) added as a supplement. PGR treatments produce biochemical and physiological changes which can modify the quantity and quality of oil production (Prins et al. 2010). The use of PGRs in agricultural practices is currently escalated because of the positive effects on the productivity and quality of agricultural products. The PGR technology can be applied in agriculture areas, where better products and yield are needed (Povh and Ono 2006). Several studies have shown that the application of PGR to some crops can increase their oil production, including in mint and aromatic oil-producing plants (Bano et al. 2016), *Mentha arvensis* L (Naeem et al. 2012), canola (Ullah et al. 2012), maize (Zhang et al. 2014), jatropha (Abdelgadir et al. 2010), soybean (Sari et al. 2018) and oil palm (Santoso et al. 2018). Since 1940, natural and synthetic plant growth regulators have been used in agriculture to regulate various processes, such as germination, vegetative growth, reproduction, maturation, leaf senescence and post-harvest. One of natural sources that contain growth regulator is seaweed extract (Sunarpi et at. 2010). Seaweed extracts are known to contain: auxin (Krajnc et al. 2013), cytokines (Mondal et al. 2015), and gibberellins (Stirk et al. 2014).

Although PGRs have been used for decades, the effects of these compounds on the production of secondary metabolites, proteins and related genes expression are not yet well known (Hussain et al. 2012). Several studies investigating biosynthetic pathways comparing plants with high productivity with those with low productivity were reported on some oil-producing plants, such as rapeseed (Li et al. 2013), soybeans (Liu et al. 2008), corn (Liu et al. 2009) and sunflower (Troncoso-Ponce et al. 2010). For this reason, this research examined the expression of important genes in oil biosynthesis in oil palm, namely ACCase, KAS III, and the stearoil enzyme ACP desaturase (FAD). In addition to genes encoded regulatory proteins were also reported to increase during oil production. To et al (2012) concluded from their research that the transcription factor WRINKLED1 (WRI1) increased its expression in line with oil biosynthesis in Arabidopsis plants. Bourgis et al. (2011) reported similar findings/regulatory gene WRI1 in oil palm and date palms. But, Sing and Cheah (2000) concluded that certain enzymes involved in fatty acid synthesis only exist or increase in significant amount in the period of oil synthesis, whereas regulatory proteins that regulate the expression of enzymes encoding genes exist near the active period of oil synthesis.

In addition to oil, oil palm also produces secondary metabolites, such as beta carotene, vitamin E and cholesterol whose biosynthesis are regulated by the

respective *phytoen synthase* (*PSY)* genes, *HGA geranylgeranyl transferase (HGGT)* and *HMG-CoA reductase*. Expression of the *ACCase* gene is important to see whether it only affects the biosynthesis of oil alone or affected also the production of other metabolites. In addition, PGRs regulate the expression of their receptor genes, including AHK3 cytokinin receptor (Caesar et al. 2011), and auxin receptor F-box protein transport inhibitor response1 (TIR1) (True et al. 2020), as well as gibberellin receptors, GID1 protein (Ueguchi-Tanaka et al. 2005). In this research, biostimulants containing PGRs from seaweed were examined for their effects on the production the primary and secondary metabolites and the expression of the regulatory genes.

MATERIALS AND METHODS

Plant materials

Fruit and leaf tissues were collected from the mature oil palm trees of Indonesia Research Institute for Biotechnology and Bioindustry (IRIBB) collection in Ciomas, Bogor, West Java, Indonesia. Biostimulant was formulated using seaweed extracts that contain plant regulator growths which are: auxin, cytokines, and gibberellins, and some amino acids and micronutrients. The treatments consist of the trunk injection of oil palm trees with 20 ml of biostimulant formula (B), 20 ml biostimulant enriched with activator of oil accumulation (BA) compared with control (C). The activator was a mixture of Mg plus Glutamate and should work on the ACCase activity of mammalian and improved oil production in oil palm (Santoso et al. 2017, Paten No P00201701786). The treatments were repeated every month for 4 months. In 4 weeks after final treatments, the samples were taken for analyses of the genes expressions and some metabolites contents (fatty acid, carotene, vitamin E, phenolic and chlorophyll).

Total RNA isolation and cDNA Synthesis

The method used for RNA extraction from fruit and leaf was described in the User Manual of RNeasy Mini kit (Qiagen). One gram of tissue, mixed with 1.5% polyvinylpyrrolidone was ground using a pestle and mortar in the presence of liquid nitrogen. After centrifugation, the pellet was washed with an equal volume of 70% cold ethanol, the ethanol was allowed to evaporate at room temperature for 15 min, and the purified RNA pellet was then resuspended in an appropriate volume of nuclease-free water. The purity and concentration of the RNA were determined spectrophotometrically at ƛ 230, 260, and 280 nm. The integrity of total RNA was checked by electrophoresis.

Synthesis of cDNA and Reverse Transcriptase Quantitative PCR (RTqPCR) setup

Analysis using Real Time qPCR used a plate with 96 wells. The PCR reaction mixture consisted of 10 µL SYBR (SYBR Hi-Rox Kit), 1 µL forward primer, 1 µL reverse primer (Table 1), 7 µL NFW and 1 µL cDNA from each dilution concentration (100, 50, 20, and 10 ng). The reaction mixture is put into 96 well plates. PCR cycle conditions consist of one denaturation cycle at 95°C for 10 minutes and followed by 45 amplification cycles (95°C for 15 seconds, 60°C for 1 minute and 72°C for 20 seconds), melt curve stage (95°C for 15 seconds, 60°C for 1 minute and 95°C for 15 seconds) and cooling at 4°C for 30 minutes. All the normalized ratios corresponding to transcript accumulation were calculated automatically by StepOne Software v2.3 provided by the manufacturer using the following calculation: Normalized Ratio = 2-Δ(Cp target-CpEgActin). Analysis Expression data were transformed into heat map presentation using Microsoft Excel 2010 (Microsoft, USA). To ensure the validity of the data, a stringent threshold of ratio value was applied. Upregulated genes were shown in bright red for a threshold value ≥5; dark red for value 0.2. The non-significant genes are shown in dark color (Putranto et al. 2016). Primers used in this research used data genomics from www.ncbi nlm.nih.gov for oil palm gene or from literature that had been confirmed the validation of primer sequence accuracy. The gene that note available for oil palm species was used the target gene from other species with the closest homology sequence.

Analysis of oil content and fatty acid composition

Mesocarp (fruit flesh) was separated from the seeds, cut into small pieces and determined the wet weight. After an overnight drying (±16 hours) in the oven, the dried samples were weighed then mashed. The samples were wrapped in filter paper, put in a tube on top of a fat flask containing benzene petroleum and mounted on a soxhlet device. After refluxed for ±8 hours or until the solvent in the soxhlet looks clear (no more oil is extracted), the fat flask was removed and dried, so that only the oil was left. Pumpkin was then weighed again. The weight of the oil is the weight of the pumpkin after soxhletation minus the weight of the pumpkin before soxhletation. The obtained oil was analyzed for its fatty acid composition by Gas Chromatography.

Vitamin E analysis: Samples of crude palm oil were dissolved in 20 mL of absolute ethanol and then spinned for 15 minutes at 3,000 rpm. After separation from precipitate, ethanol in the supernatant was evaporated. The extract was redissolved in 5 mL ethanol and analyzed using High Performance Liquid Chromatography (HPLC) with the Develosil RP Aqueous 4.6 mm column i.d. × 250 mm length. The mobile phase used is 100% methanol with a flow rate of 1 mL/min (Ng et al. 2004).

The colorimetric assay of phenolic compounds based on the reaction of FolinCiocalteu reagent is a method widely used for the determination of total phenols content in honey. The method consists of calibration with a pure phenolic compound, extraction of phenols from the sample and the measurement of absorbance after the color reaction (Gomez et al. 2006).

The chlorophyll from the fresh leaves was extracted in 80% acetone and the absorbance was read spectrophotometrically at 663 and 645 nm (Houimli et al. 2010).

Table 1. Primer pairs used in this research

Gene	Primer	References
ACCase subunit biotin carboxylase	GAAGCACCWTYCCTGCMYT CKHGTTGGRGCCAYACRAT	Budiani et al. (2004)
KAS III	CAATTATTAGATGGGGCTGAAG TATGTGACAACAGAAACCAAGC	Gu et al. 2012
Desaturase (FAD)	ACGTTACCGGAGCGTTACAC AGGAGCACCTTAATCGCTGA	*Elaeis guineensis*
AHK3	GATGGGTTGGAACGTGTTAGTC TGTTCAACACGTGGAACTACTTC	Kim et al. (2006)
TIR	CGCTGTCCAACTTCTTCCTC CGTACCATGACTTGCACACC	*Arabidopsis thaliana*
GID1	CTCCAGGACAGGGACTGGTA CATTGGACAACCTTGACGTG	*Oryza sativa*
HMG-CoA reductase	GCAACCATCAAGGAGGATGT TCCATCATTCACAGGTTCCA	*Elaeis guineensis*
Phytoen synthase (PSY)	TCCGACTGTGGCTTTCTTCT AGTCACACCAGCCTCTGCTT	*Musa acuminata*
HGA geranylgeranyl transferase (HGGT)	5′-A(C/T)(A/G)T(A/G/T/C)GT(A/G/T/C)GG(A/G/T/C)(A/C/T)T (A/G/T/C)AA(C/T)CA- 5′-GC(A/G)TA(A/G)AA(A/G/T/C) A(A/G)(A/G/T/C)TTCCA(A/G/T)AT(A/G)AA-	*Arabidopsis thaliana*
WRINKLED1	CCCGGGTATGAAGAGACCCTTAACCAC GGATCCCGACAGAATAGTTCCAAGAA	*Elaeis guineensis*
Chloropyll synthase	TCTCGTGGAATGAAGGAAGG ATCGCTGGATTACAGGCATC	*Elaeis guineensis*
Actin	TGCTGATCGTATGAGCAAGGAAA GAAATCCACATCTGCTGGAAGGT	*Elaeis guineensis*

RESULTS AND DISCUSSION

Expression of the metabolites-relating genes in fresh fruit bunches

Expression levels of the genes were varied in each biostimulant treatments. In the 4 weeks after treatment (WAT) of the biostimulant with activator (BA) sample had a similar expression value of *ACCase* (BC: *Biotin Carboxylase*) compared with Biostimulant (B), but higher values than the control samples. In the 8 and 16 WAT there was no increase in the expression value of the B and BA samples. In the 12 WAT, the B sample gave the expression value higher than the sample BA. This result indicated the high the treatment of biostimulant affected the plants in more than 4 WAT and showed no more increase more than 12 WAT (Table 2). The fold change value in sample B with respect to C was 4 WAT 5.71; 8 WAT 1.00, 16 WAT 0.01. While the fold change value in the sample BA to C was the 4 WAT 6.19; the 8 WAT 0.67; the 16 WAT 0.58.

PSY gene encoded a key enzyme in the biosynthesis of carotenoid compounds (Fuad 2010), indicating the decrease of expression value that tended to decrease in each sample given treatment. The 4, 8, and 16 WAT showed that the results of the expression values of the B and BA samples were no increase compared to the C samples. The result indicated that the treatment could not affect the expression level of PSY more than 4 WAT. *Homogentisate geranylgeranyl transferase* (HGGT) is generally believed to be the key enzyme that catalyzes the condensation of HGA and GGDP to generate tocotrienols due to the structural similarity of the tocotrienols side chain and GGDP (Munusamy and Abdullah 2019). HHGT expression level tended to decrease in some treatments. There was no increase compared to the C samples. In 12 WAT of the BA sample had higher expression value than the B.

The HMG-CoA gene serves as a catalyst in the formation of phytosterol. HMG-CoA gene expression data for B and BA samples to C samples seen from the 4 to 16 WAT indicated decreasing trends (Table 2). As plants age, the benefits of biostimulant applications decrease. The expression values in sample B with respect to C from the 4 WAT sample was 1.40, the 8 WAT sample was 0.85, and the 16 WAT samples were 0.25. While the fold change value from the sample BA to C were the 4 WAT 2.70, 8 WAT 1.21, the 16 WAT 0.65. HMG-CoA expression showed that in the 4 and 8 WAT the BA sample produced higher expression values than the B and C samples. In the 12 WAT the BA sample had a higher expression value than the B sample. In the 16 WAT there was no increase in expression of the B and BA treatment samples.

In the BA sample against the C sample from the 4 WAT to the 8 WAT the graph rises, continuing to the 16 WAT showed a decreased result. The decrease showed that the activator application increased only until the 8 WAT, then the benefits decreased. The fold change value in sample B with respect to C was 4 WAT 0.06; 8 WAT 0.21; and 16 WAT 0.54. While the fold change value in the sample BA to C was the 4 WAT 0.03; 8 WAT 0.72; and a decrease in the 16 WAT 0.51.

Table 2. *Heatmap* comparison of the expression levels of ACCase *Biotin Carboxylase, PSY, HGGT, HMG CoA gene* in 4, 8, 12, and 16 WAT

Gen	Weeks after treatments (WAT)	Expression level		
		Control ©	Biostimulant (B)	Biostimulan + Activators (BA)
BC	4			
	8			
	12			
	16			
PSY	4			
	8			
	12			
	16			
HGGT	4			
	8			
	12			
	16			
HMG CoA	4			
	8			
	12			
	16			

n ≥ 1
E-01
E-02
E-03
n≤ E-04

Table 3. Expression ratio profile four specific genes in oil palm bunches

Gen	4B vs 4C	4BA vs 4C	8B vs 8C	8BA vs 8C	16Bvs 16C	16BA vs 16C
Biotin carboksilase						
PSY						
HGGT						
HMG CoA						

N>5
1<N<5
ND
0.2<N<1
N<0,2

Note: 4B: Four weeks after treatment of biostimulant without activator. 4BA: that with activator after 4 weeks. 4C: Control after 4 weeks. 8B: Eight weeks after treatment of biostimulant without activator. 8BA: that with activator after 8 weeks. 8C: Control after 8 weeks. 16B: Sixteen weeks after treatment of biostimulant without activator. 16BA: that with activator after 16 weeks. 16C: Control after 16 weeks

Accumulation ratio of relative transcripts in oil palm fruits between the biostimulant-treated samples and the control showed that the four specific genes tested had different levels of expression between the B and BA versus C samples from the 4 to 16 WAT (Table 3). In the ACCase-Biotin Carboxylase gene, the 4C and 4BA samples gave the up-regulated results displayed in bright red, because the threshold value was equal to or greater than 5. The fold change from the 4C sample reached 5.7 and 6.2 times for the 4B and 4BA samples respectively. In the HGGT gene the expression increased only in 8BA sample as indicated in dark red and the threshold value was less than 5. The fold change of the 8BA sample was 1.3 times that of the 8C sample. The last up-regulated gene was HMG CoA in samples 4B, 4BA and 8BA. The 4B fold change of the sample was 1.4 times and the 4BA sample was 2.7 times to the 4C sample. The 8BA sample yielded 1.3 times greater than the 8C.

Down regulated results were displayed in bright green for a threshold value less than equal to 0.2. The four specific genes that produce down-regulated expressions were 8B and 16B samples in the *ACCase Biotin Carboxylase* gene, and 4B and 4BA samples in the PSY and HGGT genes. Down-regulated values were marked in dark green for values greater than 2, namely the 8BA and 16BA samples in the *ACCase Biotin Carboxylase* gene, 8B, 8BA, 16B, and 16BA in the PSY gene, 8B, 16B, and 16BA in the HGGT gene, samples 8B, 16B and 16Ba in the HMG CoA gene. Based on the results of the expression ratio showed that the *ACCase Biotin Carboxylase* and *HMG CoA* genes related to fat and phytosterol syntheses produced a maximum effect in 4 WAT after the application of the biostimulant either with or without activator into oil palm.

The pathway of the formation of unsaturated fatty acids in oil palm (*Elaeis guineensis* Jacq) begins with the activity of the FAS (Fatty acid syntase) gene (Sihombing, et al. 2019). Whereas SAD gene is a gene that functions as a determinant of the quantity of oleic which belongs to monounsaturated fatty acids (Hwang et.al. 2016). FAD and SAD genes indicated to be up regulated in B and BA samples in 4 WAT, with the addition of biostimulants without or with the activator can already trigger the expression of those genes, while the 8, 12 and 16 WAT were down regulated. In this observation the SAD gene was expressed at 4 WAT, so unsaturated fatty acids are concentrated at 4 WAT.

WRI1 is one of many contributing genes that control oil content in oil palm fruit (Baud et al. 2009). Biostimulants treatments did not trigger expression of a transcription

factor-encoded WRI1 and chlorophyll synthase genes in 4-16 WAT period (Table 4 and 5). Afterward the expression levels from both the control and treatment samples decreased. In the sample of palm leaves, meanwhile the expression value was high and increased at biostimulant treatments that trigger gene expression of KASIII (Table 4).

Expression of the metabolites-relating genes in leaves and fresh fruit bunches

AHK3 gene expression at 4 WAT was up-regulated in B and BA treatments (Table 6). Meanwhile the treatment effect showed decrease effect in 6 and 8 WAT. But in 16 WAT, B treatments showed upregulated in cytokinin and gibberellin expressions. Furthermore, results showed that samples from the treatments with biostimulant and biostimulant + activator had the highest values in the primary GID in 1 and 7 WAT, as indicated by dark brown color in the heatmap of Table 7.

Fatty acid, total carotene, vitamin E, total phenolic and chlorophyll content

Palm oil generally contains 50% saturated fatty acids, consisting of 44% palmitic acid (C16: 0), 5% stearic acid (C18: 0), and small amounts of myristic acid (C14:0). Unsaturated fatty acids are generally 40% oleic acid, 10% polyunsaturated linoleic acid (C18:2) and linolenic acid (C18:3) (Prada et al. 2011). The addition of activators increases total palmitic acid and linoleic acid (Table 8). Oil accumulation showed increase in 4 WAT and had the highest in 8 WAT. Whereas in 4 WAT, linoleic acid showed increase and meanwhile in 8 WAT, oleic acid increase. These increases have health benefits because oleic and linoleic acid are unsaturated acid leading to a reduction in cholesterol levels, atherogenesis risk blood pressure and antihypertensive (Yaqoob 2002), to induce beneficial antiinflammatory effects on autoimmune diseases and protective effect on breast cancer and improvement of immune system function (Solanas 2002).

Discussion

Jin et al. (1998) showed a very close relationship between the speed of oil accumulation and ACC activity in developing soybean seeds from various varieties with different oil contents. The expression of ACC increased in 4 and 8 WAT meanwhile it was decreased in 12 WAT in Biostimulant application but increased KASIII expression in 4 WAT. It was related to oil accumulation whereas the addition of activators increases total palmitic acid and linoleic acid (Table 8). Oil accumulation showed increase in 4 WAT and had the highest in 8 WAT. The increase of linoleic as unsaturated fatty acid that catalyzed by stearoyl-acyl carrier protein desaturase (SAD) was higher than saturated fatty acid that catalyzed by fatty acid synthase, it was related also with the higher expression level of SAD gene higher than FAS.

Table 4. Summary expression analyses of FAD, SAD, KASIII, and WR1 genes from young fresh bunches of oil palm with treatment of biostimulant (B) and biostimulan+activators (BA)

Gene	Sample name		Results
FAD	4 WAT	B	Up regulated
		BA	Up regulated
	8 WAT	B	Down regulated
		BA	Down regulated
	16 WAT	B	Down regulated
		BA	Down regulated
SAD	4 WAT	B	Up regulated
		BA	Up regulated
	8 WAT	B	Down regulated
		BA	Down regulated
	16 WAT	B	Down regulated
		BA	Down regulated
KAS III	4 WAT	B	Up regulated
		BA	Up regulated
	8 WAT	B	Down regulated
		BA	Down regulated
	16 WAT	B	Down regulated
		BA	Down regulated
WR1	4 WAT	B	Down regulated
		BA	Down regulated
	8 WAT	B	Down regulated
		BA	Down regulated
	16 WAT	B	Down regulated
		BA	Down regulated

Table 5. Expression analysis of chlorophyll synthase (CLS) from young leaves of oil palm with treatment of biostimulant (B) and biostimulan+activators (BA)

Gene	Sample name		Results
CLS	4 WAT	B	Down regulated
		BA	Down regulated
	8 WAT	B	Down regulated
		BA	Down regulated
	16 WAT	B	Down regulated
		BA	Down regulated

Table 6. Expression analysis of AHK3 and GID1 from young fresh bunches of oil palm with treatment of biostimulant (B) and biostimulan+activators (BA)

Gene	Sample name		Results
AHK3	4 weeks	B	Up regulated
		BA	Up regulated
	8 weeks	B	Down regulated
		BA	Down regulated
	16 weeks	B	Up regulated
		BA	Down regulated
GID1	4 weeks	B	Down regulated
		BA	Down regulated
	8 weeks	B	Down regulated
		BA	Down regulated
	16 weeks	B	Up regulated
		BA	Down regulated

Table 7. Expression analyses of transcription factor of oil synthesis WR1, gibberellin GID and cytokinin AHK

Primer	Weeks after treatments (WAT)	B leaves	BA leaves	Control fruits	BA fruits
AHK	1				
	7				
GID	1				
	7				
WR1	1				
	7				

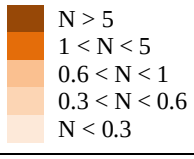

Table 8. Fatty acid composition of ripen mesocarps from B, BA and C in 4, 8 and 12 WAT

Fatty acid composition	Average fatty acid composition (%)								
	4 WAT			8 WAT			12 WAT		
	C	B	BA	C	B	BA	C	B	BA
Fat	65.52	66.30	65.91	48.06	53.67	50.34	32.02	27.81	24.88
Lauric acid C-12:0	0.06	0.04	0.05	0.04	0.02	0.04	0.02	0.03	0.07
Miristic acid C14:0	0.54	0.64	0.59	1.08	0.74	0.74	0.45	0.44	0.80
Pentadecanoaic acid C15:0	0.02	0.02	0.02	0.04	0.02	0.04	0.03	0.03	0.05
Palmitic acid C16:0	37.91	36.06	36.98	43.41	39.36	41.64	34.65	37.23	38.06
Palmitoleic acid C16:1	0.08	0.08	0.08	0.11	0.08	0.09	0.06	0.07	0.10
Heptadecanoic acid, C17:0	0.08	0.08	0.08	0.08	0.09	0.09	0.07	0.09	0.09
Cis-10-heptadecanoic acid, C17:1		0.02	0.02						
Stearic cid, C18:0	4.14	4.475	4.31	4.68	5.30	4.44	4.81	4.91	3.63
Oleic acid, C18:1n9c	36.65	36.83	36.74	35.53	38.94	38.11	41.67	43.40	35.43
Linoleic acid, C18:2n6c	9.74	10.64	10.19	12.08	12.64	10.56	11.50	12.60	13.57
Asam arachidic, C20:0	0.29	0.31	0.30	0.32	0.35	0.30	0.39	0.36	0.32
Linolenic acid, C18:3n3	0.41	0.40	0.40	0.46	0.37	0.41	0.35	0.12	0.08
cis-11, 14-eicosedienoic acid, C20:2	0.04	0.04	0.04	0.04	0.07	0.06	0.09	0.38	0.40
Behemic acid, C22:0	0.06	0.06	0.06	0.06	0.06	0.05	0.07	0.06	0.05
Lignoceric acid, C24:0	0.04	0.04	0.04	0.06	0.04	0.06	0.06	0.06	0.06
Fatty acids	90.07	89.71	89.89	97.96	98.16	96.61	94.36	99.83	92.76

Note : BA: Biostimulant with activators, B: Biostimulant with no activator, C: Control

PGRs from seaweed extracts affected the production of secondary metabolites, proteins and the expression of the related genes in oil palm. Meanwhile carotene production showed no significant difference (Table 9) and it was related to the expression of PSY gene that down regulated (Table 4). According Rasid et al (2014), phytoene desaturase (PDS) which involves in carotein synthesis, was shown to be relatively high in the young mesocarp tissue (5 weeks) and however, the expression decreased to about onefold from 7th weeks to 13th weeks. The expression further decreased to the lowest level in the 15 week after anthesis (WAA), but the expression then was increased to about two-fold in 17 and 19 WAA tissues.

However, the HGGT showed preferential expression in the oil-bearing mesocarp and kernel tissues and was highly expressed at the fruit ripening stage of the mesocarp supporting its role in providing protection to the vegetable oils from oxidative damage (Kong et al. 2016). Expression data of HGGT gene is similar to the PSY gene, in sample B against sample C seen from the 4 to the 16 WAT showed an up regulated result (Table 2xx). In the BA sample against the C sample from the 4 to the 8 WAT the graph rises, continuing to the 16 WAT showed decreasing result. The fold change value in sample B to C was 0.03 in 4 WAT, 0.25 in 12 WAT and 0.56 in 16 WAT. Sample BA to C was 0.01; up in the 12 WAT 1.35 and down in the 16 WAT 0.98. A significant increase was shown in the addition of activators compared to biostimulants alone. In the HGGT gene was more appropriate induced with the addition of activators to get maximum metabolites. These research data indicated that the addition of activators (BA) increases the expression of HMG CoA genes compared to the addition of biostimulant without activator (B).

WR1 showed down-regulated expression in 4-12 WAT. In assumed that the induction it more early period of application and it showed that the expression level was increased in 1st week of application in the leaves samples. It is also for the hormone expression whereas GID1. According to Sivaramakrishnan et al. (2020), PGR showed the highest stimulatory effect on the omega-3 fatty acids content, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Meanwhile cells treated with ABA had the highest lipid in *Chorella* sp. The elevated levels of saturated fatty acids (C16:0 and C18:0) were consistent with high expression of the ketoacyl ACP synthase (KAS) gene. However, the increment of stearoyl-ACP desaturase (SAD) gene expression upon IAA induction coincided with oleic acid (C18:1) production. Application of gibberelin in culture medium was shown to promote transient increment of palmitic (C16:0) and stearic (C18:0) acids and these

Table 9. Protein and vitamin E concentration in the oil palm mesocarps

Treatments	Protein content in young oil palm mesocarp (mg/g)			Vitamin E content of palm oil (mg/100 g)			Total carotenenoid of ripe oil palm mesocarp (mg/L)	
	4 WAT	8 WAT	12WAT	4 WAT	8 WAT	12 WAT	4 WAT	8 WAT
Control (C)	9.49	29.90	29.90	29.90	52.35	5.57	863.52	985.63
Biostimulant (B)	17.96	89.30	89.30	89.30	56.20	4.34	884.30	986.44
Biostimulant with activators (BA)	12.10	13.68	79.53	67.35	63.35	8.86	764.41	985.14

Table 10. Chlorophyll and phenolic contents of the oil palm leaves

Treatments	Chlorophyll contents (mg/L)			Phenolic contents (mg/L)		
	4WAT	8WAT	12WAT	4WAT	8WAT	12WAT
Control (C)	76.10	119.43	182.28	41.03	44.99	40.54
Biostimulant (B)	65.62	132.92	186.39	39.61	41.12	36.94
Biostimulant with activators (BA)	75.82	107.96	218.49	40.08	42.94	41.82

changes are correlated with the expression of β-*ketoacyl ACP synthase* (*KAS*) gene (Jusoh et al. 2019). For protein content, the application has the potential to increase protein in young fruits and increase vitamin E levels, while total carotene was not too significantly different (Table 9). Moreover, the treatments tend to increase the chlorophyll content, while the phenolic content was not significantly different (Table 10). Cao et al. (1997) showed that application of GA3 increased the growth, protein secretion, and starch accumulation in maize endosperm suspension cells.

In conclusion, application of plant biostimulants with or without activator for oil accumulation into oil palm through trunk injection indicated to increase expression of the genes regulating oil synthesis and the related metabolites. The expression ratio of the *ACCase Biotin Carboxylase* and *HMG-CoA* genes related to fat and *phytosterol syntheses* were maximum in 4 WAT (weeks after treatment) of biostimulant with activators and 8 WAT of biostimulants without activator. In addition, the expression of the gibberellin (GID) gene was highest in 1 and 7 WAT with biostimulant added activator and biostimulant alone. The addition of activators increased total palmitic acid and linoleic acid contents. Oil accumulation showed to increase in 4 WAT dan had highest in 8 WAT of the biostimulant with activator.

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