J Plant Biotechnol (2023) 50:127-136
DOI:https://doi.org/10.5010/JPB.2023.50.016.127

ISSN 1229-2818 (Print)
ISSN 2384-1397 (Online)

Research Article

Chlorophyll contents and expression profiles of photosynthesis-related genes in water-stressed banana plantlets

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Received: 12 March 2023 / Revised: 1 May 2023 / Accepted: 23 May 2023 / Published: 22 June 2023

Abstract Water scarcity decreases the rate of photosynthesis and, consequently, the yield of banana plants (*Musa* spp). In this study, transcriptome analysis was performed to identify photosynthesis-related genes in banana plants and determine their expression profiles under water stress conditions. Banana plantlets were in vitro cultured on Murashige and Skoog agar medium with and without 10% polyethylene glycol and marked as BP10 and BK. Chlorophyll contents in the plant shoots were determined spectrophotometrically. Two cDNA libraries generated from BK and BP10 plantlets, respectively, were used as the reference for transcriptome data. Gene ontology (GO) enrichment analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) and visualized using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway prediction. Morphological observations indicated that water deficiency caused chlorosis and reduced the shoot chlorophyll content of banana plantlets. GO enrichment identified 52 photosynthesis-related genes that were affected by water stress. KEGG visualization revealed the pathways related to the 52 photosynthesis-related genes and their allocations in four GO terms. Four, 12, 15, and 21 genes were related to chlorophyll biosynthesis, the Calvin cycle, the photosynthetic electron transfer chain, and the light-harvesting complex, respectively. Differentially expressed gene (DEG) analysis using DESeq revealed that 45 genes were down-regulated, whereas seven genes were up-regulated. Four of the down-regulated genes were responsible for chlorophyll biosynthesis and appeared to cause the decrease in the banana leaf chlorophyll content. Among the annotated DEGs, *MaPNDO*, *MaPSAL*, and *MaFEDA* were selected and validated using quantitative real-time PCR.

Keywords *Musa acuminata* Colla, Photosynthesis, Transcriptome analysis, Water deficiency

Introduction

Bananas (*Musa* spp) are considered as one of major food crops since it is providing a substantial source of nutrition and food security in the world. Bananas are originated and grown well in Southeast Asia, including Indonesia. Majority of cultivated banana cultivars have arisen primarily as a result of hybridizations between *M. acuminata* ('A'-genome) and *M. balbisiana* ('B'-genome) which naturally resulted in various combinations of these two genomes. Most edible fruit bananas are parthenocarpy and triploids (Davey et al. 2013; Nayar 2010) with genome constitutions of AAA (dessert banana), AAB (plantains) and ABB (cooking bananas).

Banana plants are known to suffer against water deficiency, although the drought tolerance response in banana is genotype-dependent (Nansamba et al. 2020; Ravi et al. 2013). There was a tendency that banana varieties with more 'B' genome are more tolerant to drought stress compare to the varieties with more 'A' genome (Wang et al. 2020). Studies were reported to discover effects of water stress to the disruption of physiological and biochemical processes in banana. Water deficiency is known to alter the normal condition of banana plants and causes the morphological and physiological changes. Many reports showed that to prevent water loss, tolerant banana plants

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made various morphological adjustments, such as leaf rolling, root lengthening, leaf size reduction, and leaf abscission. Changes also occurred in the cellular level, including changes in metabolic pathway directions, nutrient and ion uptakes, synthesis of new proteins or modulation of free radical generation (Surendar et al. 2013a; Wang et al. 2020).

To improve understanding about the molecular mechanism of banana plants to respond water stress, some studies have been accomplished using the next generation sequencing (NGS) technology. Transcriptomic analysis has become a reliable approach to unravel the integrated insight into abiotic stress tolerance mechanisms occurred in banana plants (Backiyarani et al. 2015; Hu et al. 2017). Transcriptomic analysis approach was able to discover enriched differentially expressed genes (DEGs) from cDNA libraries of the tolerant and sensitive banana cultivars that exposed to drought (Muthusamy et al. 2016). In drought-stressed banana, identified DEGs were mainly involved in important bioprocesses, such as lipid metabolism, carbohydrate degradation, protein modifications, alkaloid biosynthesis and other secondary metabolites. Major biological processes were known to be influenced by water deficiency, including photosynthesis, cellular respiration, responses to stress, and organ development.

Photosynthesis seems to be the most altered by drought and is very closely related to banana growth and fruit production (Surendar et al. 2013b). Impacts of water stress on photosynthesis have been studied based on molecular perspective in various plant species. Studies discovered disturbances of water deficit condition on photosynthesis and successfully identified some responsive genes involved in photosynthesis. Water scarcity has been known to decrease not only the photosynthesis rate, but also destruct the photosystem II, causing the decline of light capture capacity (Sasi et al. 2018). It was suggested that water-stress caused stomata closure, decreases stomata conductance and resulting in diverting electrons from the photosynthetic electron transport chain to molecular oxygen generating ROS at the end of photosystem I (Chen et al. 2020). It was reported that water shortage defected the protein structures of oxygen-evolving complex of PSII and PSI reaction centers (Dalal and Tripathy 2018). However, further studies are still needed to identify the potential genes for developing DNA based markers in the future. This study was aimed to identify photosynthetic related genes of water stressed banana plantlets and determining its expression profiles based on transcriptional analysis. A transcriptome dataset was generated from cDNA libraries of banana plantlets, *Musa acuminata* cv Barangan Merah, which is one of the most popular banana cultivars in Indonesia that producing nutritive banana fruits (Sebayang et al. 2018). Nevertheless, as is common in *M. acuminata* species with A genome constitution, Barangan Merah banana is susceptible to water shortage and greatly reduces the fruit production as the impacts of insufficiency water supply. Hence, it is worthwhile to understand how severely the water stress induced alterations on photosynthesis in banana cv Barangan Merah using a molecular approach. The transcriptome dataset used in this study has been registered and submitted at the NCBI BioProject database (BioProject ID PRJNA970186).

Materials and Methods

Plant Materials

Plantlets of banana, *M. acuminata* cv 'Barangan Merah' were used in experiments as the source of samples for chlorophyll content determination and confirmation of gene expressions. Plantlets were cultured and in vitro regenerated from axillary shoots on MS agar medium (Murashige and Skoog 1962) which was supplemented with 5 μM 6-Benzylaminopurine (BA). Shoots were sub-cultured every four weeks and used as explant sources for water stress treatments. To induce water deficiency condition, 10% polyethylene glycol (PEG-6000) was added into culture medium of the BP10 shoot cultures. Shoots grown on culture medium without PEG were used as the control treatment (BK). Shoots were regenerated into plantlets in four weeks. Banana in vitro cultures were placed in a controlled room at 22 ± 2°C and continuous-lighting. Six replicates were made for each treatment. After a four-week period of PEG treatments, BK and BP10 plantlets were collected for chlorophyll and RNA extractions.

Determination of Chlorophyll Content

Shoot parts were detached from plantlets of the BK and BP10 after a four-week PEG treatment and prepared for chlorophyll extraction. As much as 0.1 g shoot tissues grinded and added with two ml 96% ethanol in 4-6 minutes until all the chlorophyll was dissolved (Wintermans and De Mots, 1965). Crude extracts were filtered with filter paper Whatman No.1. Chlorophyll contents were measured using a spectrophotometer (BioRad SmartSpec^{TM}Plus) at 649 nm and 665 nm wavelengths.

Transcriptomic Analysis for GO Enrichment and DEGseq Analysis

A transcriptome dataset obtained from previous experiments was used in this study as a reference data (Widiyanto et al. 2019, unpublished report) and as it was mentioned that the sequences of transcriptome data was registered at the NCBI BioProject database. Two cDNA libraries of banana (*M. acuminata* cv 'Barangan Merah') were generated and constructed from plantlets of BK (control) and the highest-level stress treatment with the addition of 10% PEG (BP10). Transcriptomic analysis was performed to identify photosynthetic related genes and visualize its prediction pathways. The cDNA library generated from BP10 was subjected to be annotated for gene ontology (GO) enrichment and the cDNA library of BK was used to normalize. To annotate photosynthesis-related genes, the GO enrichment was accomplished using the database of DAVID (Database for Annotation, Visualization, and Integrated Discovery). The KEGG (Kyoto Encyclopedia of Genes and Genome) was applied to visualize photosynthetic-related gene pathways of the banana plantlets (BP10) under 10% PEG-induced water stress. The read-count data of photosynthesis-related genes were analyzed using DESeq package for determining relative expressions of annotated genes. The gene expressed numbers were normalized to the number of transcripts per million (TPM) tags (Love et al. 2014). Each unigene was grouped based on the ratio of TPM to that of the control data and further analyzed with a cut-off ratio of log10 > 2 to obtain the list of unigenes with multiples of more than 100 times compared to those of the control group (p-value $\leq$ 0.001).

Validation of Gene Expressions

To confirm the expressions of selected DEGs, an independent experiment was conducted to replicate a similar experiment with previous experiments when the cDNA libraries of transcriptome data were constructed. The total RNA samples were isolated from in vitro shoot cultures of the BK and BP10 banana cv Barangan Merah plantlets after four weeks of the PEG treatment. Three genes i.e., *MaFEDA (2Fe-2S ferredoxin)*, *MaPSAL (photosystem I subunit l)* and *MaPYROXD1 (pyridine nucleotide-disulfide oxidoreductase)* were selected from the annotated DEGs list (Supplementary Table S.1) and subjected to qRT-PCR assay for validation of gene expressions. Primer pairs (Table 1) were designed using the Primer3Plus package, checked with the Primer Blaster, and compared to that of the banana genome available at CIRAD with the *M. acuminata* genome. Gene expression validation was performed using the qRT-PCR assay procedure. Total RNA samples were isolated from shoots tissues using the CTAB method (Kusdianti et al. 2016). The cDNA was constructed using the GoScript™ Reverse Transcription System according to manufacture manual (Promega, USA). Three technical replicates were applied in the qRT-PCR assay. Gene expression data were normalized with two housekeeping genes, the *MaACT* and *MaBT*. Relative expression levels were determined using the $2^{-\Delta\Delta CT}$ method (Hu et al. 2017; Livak and Schmittgen 2001).

Results

Morphological Changes and Total Chlorophyll Content

Morphological observation indicated that plantlets of banana cv Barangan Merah regenerated from 10% PEG-exposed shoots (BP10) were turned pale and chlorosis after four weeks of culturing (Fig. 1). Leaves of plantlets were obviously experienced reduction of the normal growth and green coloration. In line with the morphological changes, chlorophyll determination implied that water deficiency caused a considerable reduction in total chlorophyll content (Fig. 2). Compare to that of the control plantlets (BK),

Table 1 The primer sequences used for amplifying the selected (*MaFEDA, MaPSAL*, and *MaPYROXD1*), and housekeeping genes

Symbol	Gene name	Forward primer	Reverse primer	Amplification length (bases)
MaACT	Actin *(housekeeping gene)*	CTGACTGGCAGCAGGACATA	CCAAATCGTGCCTTTGAACT	162
MaBT	Betatubulin *(housekeeping gene)*	AGTCCGGAGCTTCAACCTTT	ACGCTGACGATGGAGAAGAC	221
MaFEDA	2Fe-2S ferredoxin	TTGCCATCTCTCCCTGTCTT	GGCATTCGATCACCTTCTCT	214
MaPSAL	photosystem I subunit l	GCATCTCACGAACACCATTG	GATGGGCTGAATCACTTGGT	196
MaPYROXYD1	Pyridine nucleotide-disulfide oxidoreductase	GCTTTCTCCAGCATCAAAGG	CCCATTCCTCCTTCGACATA	216

total chlorophyll contents of the BP10 plantlets, as well as chlorophyll a and chlorophyll b were obviously decrease. It seemed that water deficiency experienced in banana plantlets inhibited its growth and chlorophyll biosynthesis, particularly in shoot parts.

Gene Ontology Enrichment of Photosynthetic Genes

The GO enrichment was completed using DAVID and identified 52 photosynthesis-related genes that were affected by water stress in BP10 plantlets Supplementary Table S.1. The 52 photosynthetic genes were distributed in four GO terms i.e., four genes in chlorophyll biosynthesis, twelve genes in Calvin cycle pathway, fifteen genes in photosystem-electron transfer chain (PETC), and twenty-one genes in antenna light harvesting complex proteins (Table 2). The 52 identified genes were among the 100 annotated genes that either the most downregulated or upregulated in PEG treatments (PEG 2.5, PEG7, and PEG10) and listed in Supplementary Table S.2-S.3. The KEGG prediction pathway was applied to visualize the photosynthesis-related gene allocations.

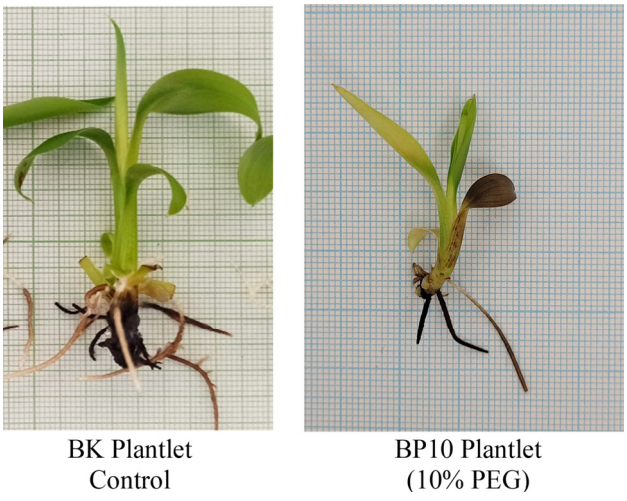

Fig. 1 Banana cv Barangan Merah plantlets cultured for four weeks in Murashige and Skoog agar medium (a) without (BK; control) and (b) with (BP10) 10% polyethylene glycol

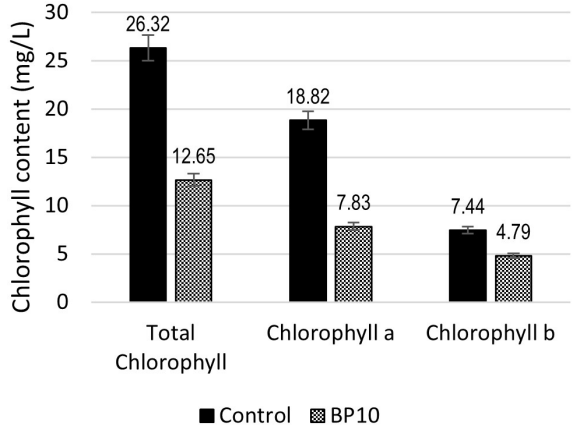

Fig. 2 The total chlorophyll, chlorophyll a, and chlorophyll b contents of shoots of the control (BK) and 10% PEG-treated (BP10) plantlets

Changes in Gene Expressions in Chlorophyll Biosynthesis

Based on transcriptomic analyses there were four genes in chlorophyll biosynthesis that affected by water stress (Table 3) and mapped into KEGG pathway (Supplementary Data Fig. S.1). The four genes are *MaPORA, MaPORB, MaHEMA1*, and *MaPYROXD1*, each of them encoded for the enzyme of synthesis of protochlorophyllide oxidoreductase-A, the enzyme of protochlorophyllide oxido-reductase-B, the family protein of glutamyl-tRNA reductase, and the family protein of pyridine nucleotide-disulfide oxidoreductase. Prediction pathway of KEGG showed that the four affected genes were down-regulated and lowering gene expressions in porphyrin and chlorophyll metabolism. Changes in gene expressions seemed to be related with the decrease of chlorophyll content in BP10 plantlets.

Alterations in Calvin Cycle

The Calvin cycle has three main phases, those are the carbon fixation, the reduction of carbon dioxide and regeneration of carbon dioxide acceptor phases. There were 12 identified genes in Calvin cycle that altered by water stress and were mapped into KEGG pathway. The alteration of 12 genes were illustrated in Supplementary Fig. S.2 and listed in Table 4. Based on KEGG pathway, there were seven genes up-regulated and five genes down-regulated. Water deficiency was down-regulated four genes of the 3-PGA biosynthesis in reduction phase and five genes in the RuBp regeneration phase. Water stress also interfered the expressions of transketolase (2.2.1.1) and aldolase superfamily protein (4.1.2.13) genes.

Table 2 The number of photosynthesis-related genes in water-stressed banana plantlets (categorized under four GO terms based on DAVID)

GO terminology	Numbers of genes
Biosynthesis of chlorophyll	4
Calvin cycle	12
Light-harvesting complex	15
Photosynthetic electron transfer chain	21
Total photosynthetic genes affected	52

Table 3 List of the four genes related to porphyrin and chlorophyll metabolism whose expressions were altered under water stress (according to the KEGG prediction pathway)

Enzyme code	*Musa* ID	Symbol	Gene name (encoded protein)
1.3.1.33	Ma11_p01810.1	*MaPORA*	*protochlorophyllide oxidoreductase A*
	Ma03_p14780.1	*MaPORB*	*protochlorophyllide oxidoreductase B*
1.2.1.70	Ma02_p00210.1	*MaHEMA1*	*glutamyl-tRNA reductase family protein*
1.3.1.111	Ma08_p24890.1	*MaPYROXD1*	*pyridine nucleotide-disulfide oxidoreductase family protein*

Table 4 List of the twelve Calvin cycle-related genes whose expressions were altered by 10% PEG; seven genes were up-regulated and five genes were down-regulated

Enzyme code	*Musa* ID	Symbol	Gene name (encoded protein)
Up-regulated:			
1.1.1.37	Ma05_p03680.1	*c-NAD-MDH2*	*lactate or malate dehydrogenase*
1.2.1.12	Ma06_p01470.1	*GAPC2*	*glyceraldehyde-3phosphate dehydrogenase C2*
	Ma06_p01470.1	*GAPCP-1*	*glyceraldehyde-3-phosphate dehydrogenase of plastid 1*
2.6.1.2	Ma11_p08770.1	*ALAAT2*	*alanine aminotransferase 2*
4.1.2.13	Ma06_p11050.1	*PDE345*	*aldolase superfamily protein (PDE345)*
	Ma05_p27790.1	*FBA8*	*aldolase superfamily protein (FBA8)*
	Ma05_p27790.1	*FBA6*	*aldolase superfamily protein (FBA6)*
Down-regulated:			
1.2.1.13	Ma10_p12550.1	*GAPA*	*glyceraldehyde 3-phosphate dehydrogenase A*
	Ma11_p20650.1	*GAPB*	*glyceraldehyde-3-phosphate dehydrogenase B*
2.2.1.1	Ma03_p16840.1	*TKL2*	*transketolase*
2.7.1.19	Ma05_p03450.1	*PRK*	*phosphoribulo kinase*
2.7.9.1	Ma03_p26110.1	*PPDK*	*pyruvate orthophosphate dikinase*

Disturbances to Antenna and Light Harvesting Complex (LHC) Proteins

Light harvesting is the first subprocess in light-dependent reactions occurs in antenna protein complexes located in photosystems on thylakoid membranes. There were 15 genes related to light harvesting complex and antenna proteins that affected by 10% PEG treatment which were mapped into KEGG pathway (Table 5). The KEGG map showed the allocations of 15 genes in the light harvesting complex (LHC) and antenna (Supplementary Fig. S.3). Identified genes consisted of 5 light harvesting complex-a (Lhca) genes located in photosystem I and 10 light harvesting complex-b (Lhcb) genes in photosystem II. It was indicated that photosystem-II was more sensitive and had more impacts by water stress than photosystem- I.

Disruptions to Photosystem Electron Transfer Chain (PETC)

Genes related to photosystem-electron transfer chain (PETC) that disrupted by water deficiency were mapped into KEGG pathway (Fig. 3) and listed in Supplementary Table S.1. The water stress condition was down-regulated a total of 21 genes in photosystem-electron transfer chain (PETC) that might cause changes of excitation rate of electrons. These genes composed of a gene encodes enzyme 1.18.1.2 *(FNR2)*, two genes encode carrier proteins (*DRT112* and *FED A*), ten genes encode photosystem I subunits, and eight genes encode photosystem II subunits (Fig. 3). Water deficiency was also disrupted the *PSBO* (oxygen evolving complex-1) gene, the component of photosystem II and known to play a role in water photolysis. Prediction pathway of KEGG showed that the light energy that captured by the antenna protein will be passed to the reaction center and excited the electron to a higher energy level (Fig. 3).

Verification of Selected DEGs

The confirmation of gene expressions showed that compared to the control plantlet (BK), the relative expressions of *MaFEDA*, *MaPSAL*, and *MaPNDO* genes were obviously altered by PEG-induced water stress in BP10 plantlets (Fig. 4). Based on its relative expression values, the *MaFEDA* and *MaPYROXD1* genes were down-regulated, whereas *MaPSAL* was up-regulated as responses to water stress.

Table 5 List of the 15 genes related to the antenna or light-harvesting complex (LHC) whose expressions were altered under water stress; according to the KEGG pathway, there were five light-harvesting complex-a *(Lhca)* genes located in photosystem I and 10 light-harvesting complex-b *(Lhcb)* genes in photosystem II

KEGG Code	*Musa* ID	Symbol	Gene name (encoded protein)
Lhca1	Ma02_p08270.1	*LHCA1*	*chlorophyll a-b binding protein 6*
Lhca2	Ma06_p22130.1	*LHCA2*	*photosystem I light-harvesting complex protein*
Lhca3	Ma03_p03930.1	*LHCA3*	*PSI type III chlorophyll a/b-binding protein*
Lhca4	Ma02_p16740.1	*LHCA4*	*light-harvesting chlorophyll-protein complex I s.u A4*
Lhcb1	Ma10_p15370.1	*CAB1*	*chlorophyll A/B binding protein 1*
	Ma02_p11170.1	*LHB1B1*	*light-harvesting chlorophyll-protein complex II-B1*
	Ma10_p15370.1	*LHB1B2*	*photosystem II LHC protein-B1B2*
Lhcb2	Ma04_p39550.1	*LHCB2.1*	*photosystem II light-harvesting complex protein 2.1*
	Ma04_p39550.1	*LHCB2.2*	*photosystem II light-harvesting complex protein 2.2*
	Ma02_p11170.1	*LHCB2.3*	*photosystem II light-harvesting complex protein 2.3*
Lhcb3	Ma09_p21570.1	*LHCB3*	*light-harvesting chlorophyll B-binding protein 3*
Lhcb4	Ma08_p03640.1	*LHCB4.1*	*light-harvesting complex photosystem II*
	Ma09_p02760.1	*LHCB4.2*	*light-harvesting complex photosystem II*
Lhcb5	Ma06_p14120.2	*LHCB5*	*light-harvesting complex of photosystem II 5*
Lhcb6	Ma07_p20600.1	*LHCB6*	*light-harvesting complex photosystem II subunit 6*

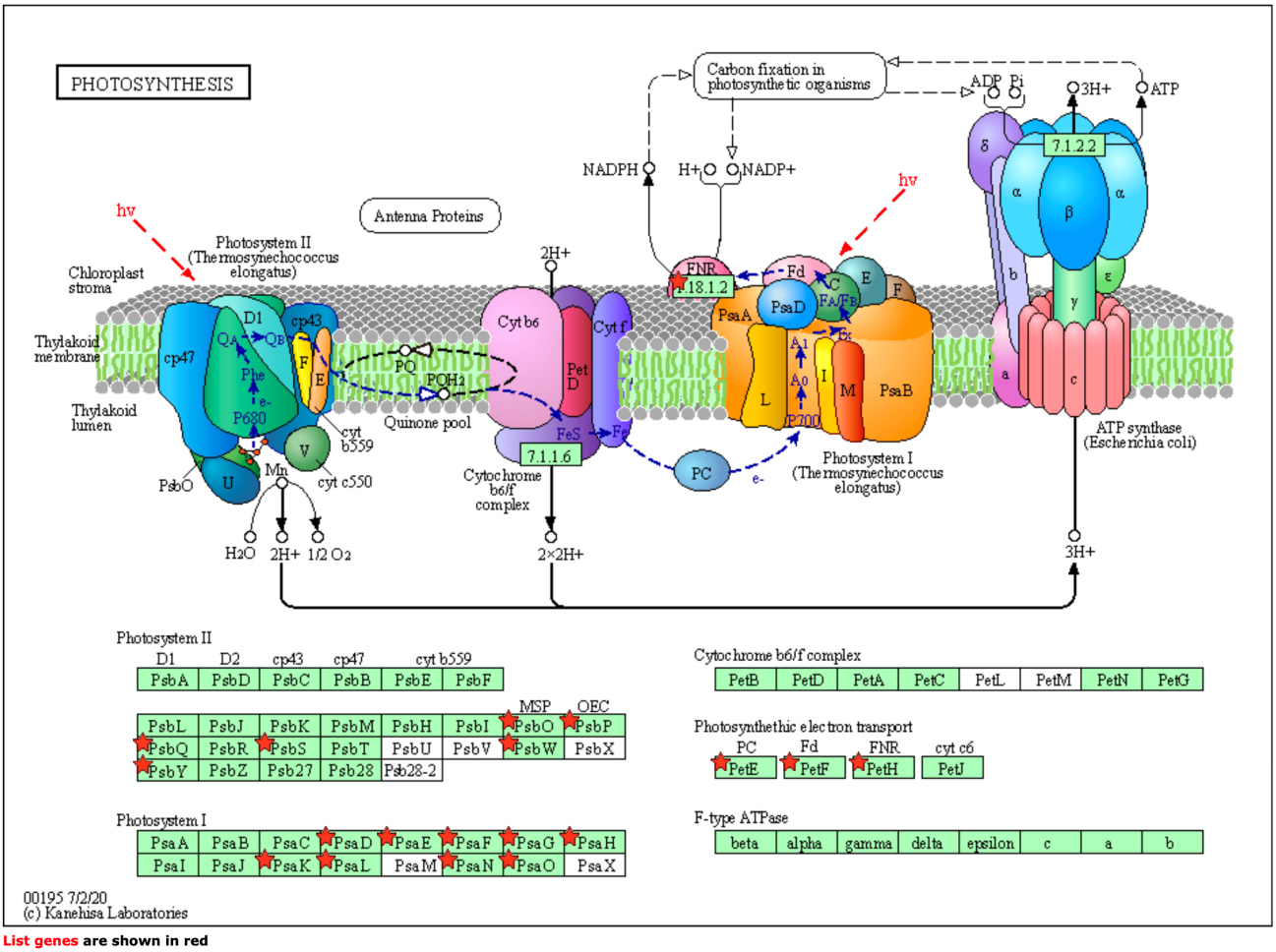

Fig. 3 Water stress-induced changes in the photosynthetic electron transfer chain (PETC) of BP10 banana plantlets. A total of 21 genes were down-regulated (marked with red stars; listed in Supplementary Table S.1)

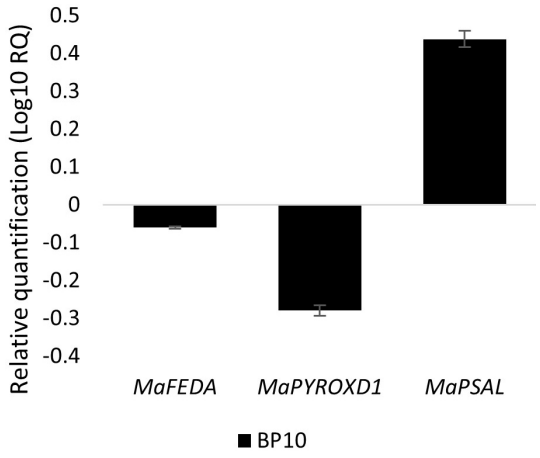

Fig. 4 The RT-qPCR-based expression profiles of *MaFEDA, MaPYROXD1*, and *MaPSAL* genes in the shoots of BP10 plantlets

Discussion

Water deficiency is a critical factor for the growth of banana plants and causing substantial changes in its growth, morphological features and biochemistry reactions. Chlorosis is the condition of plant tissues, especially in shoots or leaves that lost their green color and turn pale as the result of lack of chlorophyll pigment (Surendar et al. 2013b). The chlorosis occurred in leaves of 10% PEG-exposed plantlets seemed to be correlated with the disruption in chlorophyll biosynthesis process. Chlorosis in banana leaves seemed to be occurred because of the deterioration of chlorophyll and other photosynthetic pigments (Vergeiner et al. 2013). Water shortage is also known to cause the disruption of ultrastructural feature of chloroplasts in teak (Galeano et al. 2019). The transcriptomic analysis in this study would be able to discover the reason.

Water stress in banana plantlets caused the decrease in chlorophyll content up to 52% (Fig. 2). A considerable reduction in total chlorophyll contents was also noticed in banana plants under water shortage condition in the field (Surendar et al. 2013b). Water stress decreased total chlorophyll content and declined yield of banana production. Chlorophyll biosynthesis is consisting of a biochemical reaction series and catalyzed by numerous enzymes as illustrated in Supplementary Fig. S.1. At least there are three distinct phases in chlorophyll biosynthesis. The first phase begins from glutamic acid metabolism pathway, including the heme biosynthesis pathway to synthesize protoporphyrin. Glutamyl-tRNA reductase family protein (1.2.1.70) enzyme encoded by the *HEMA1* gene that catalyze early stages of heme biosynthesis. The second phase is the chlorophyll-a (Chla) synthesis and the photoreduction of protochlorophyllide (Pchlide) to produce chlorophyllide (Chlide) which is catalyzed by protochlorophyllide oxidoreductase (POR) enzyme. The POR proteins are the essential enzymes in chlorophyll biosynthesis, including PORA and PORB and PORC proteins (Zhao et al. 2020). The third phase is the interconversion of Chl a and chlorophyll b (Chl b) which is catalyzed by chlorophyllide an oxygenase (CAO).

As it was mentioned, the reference transcriptome data used in this study was obtained from previous experiments (Widiyanto et al. 2019, unpublished report). The transcriptome dataset was generated from four cDNA libraries of banana plantlets using Illumina MiSeqTM 2000 platform. In experiments, banana plantlets were in vitro regenerated from shoot-buds of *M. acuminata* cv Barangan Merah. Polyethylene glycol (PEG-6000) at 2.5% (BP2), 7.5% (BP7), and 10% (BP10) was supplemented to induce a water shortage condition. The control (BK) plantlets were grown without PEG. Transcriptome profiling was achieved through a bioinformatics analysis flow, including the assembly, the estimation of gene abundance, the annotation, the differential expressed genes analysis, and the gene ontology (GO) enrichment. Transcriptomic profiles indicated there were 3,351 genes altered by water stress which were covering 113 terminologies in biological processes. Statistical analysis identified 1,744 genes as differentially expressed (DEGs) under PEG treatment in which 1,046 genes of them were mapped to the reference genomes, *Musa acuminata* cv Pahang at CIRAD. These DEGs were distributed in 26 functional clusters. Among those genes, 233 annotated genes, were consistently downregulated in all PEG treatments and 100 genes of them were listed in Supplementary Data Table S.3. Conversely, 762 genes were persistently upregulated. A total of 100 genes with the highest expression level that enhanced by at least thousand times were listed in Supplementary Data Table S.4. However, transcriptomic analysis has not been thoroughly unraveled to discover the impacts of water stress treatments to any major bioprocess in details, specifically photosynthesis. In this study, further transcriptomic analysis was directed to reveal how water stress altered and disrupted particularly in photosynthesis pathway and its apparatus. The highest level of stress (10% PEG) was taken into consideration as it seemed to generate severe impacts on banana plantlet's growth.

Correspondingly with the results of this study, KEGG mapping showed that in BP10 plantlets, water stress down-regulated the *MaPORA, MaPORB*, and *MaHEMA1* genes and caused to depress the biosynthesis of its encoded enzymes. Changes in level expressions of enzymes in porphyrin and chlorophyll metabolism seemed to cause the decrease of chlorophyll biosynthesis in BP10 plantlets.

Water stress was also down-regulated the MaPYROXD1 gene that is known to play a role in the biosynthesis of chlorophyll and also involved in terpenoid backbones biosynthesis in plant cells. Alterations of gene expressions that responsible for chlorophyll biosynthesis seemed to cause the decrease of chlorophyll content in leaf tissues of banana plantlets exposed to PEG. Similar result in rice seedling and potato that drought stress also changed the expressions of *PORA* and *PORB* genes (Chen et al. 2020; Nguyen et al. 2021).

The three main stages of Calvin cycle reactions are connected processes to each other. Changes in expression levels of the *glyceraldehyde-3-phosphate dehydrogenase* (1.2.1.12 and 1.2.1.13) genes at the 3-phosphoglyceric acid (PGA) reduction phase will affect the formation of the *glyceraldehyde-3-phosphate* (GAPC), which can interfere the regeneration process of ribulose biphosphate (RuBP) and consequently will disturb the formation of glucose. Changes the expression levels of glyceraldehyde-3-phosphate dehydrogenases (1.2.1.12 and 1.2.1.13) genes seemed to affect four of the six stages of RuBP regeneration, and hence will disrupt the CO_2 fixation process. Although drought stress did not affect gene expression related to CO_2 fixation directly, but changes in gene expression related to RuBp regeneration will also interfere the CO_2 fixation process. Comparably, in rice seedling, the addition of PEG also declined the efficiency activity of Rubisco and reduced the activity of other enzymes of the carbon fixation pathway. Further analysis revealed the down-regulation of proteins involved in carbon reduction reactions, such as rubisco, triose-phosphate-isomerase (TPI), fructose 1,6-bisphosphate aldolase, sedoheptulose-1, 7-bisphophatase, and phosphoglycerate kinase (Dalal and Tripathy 2018).

In potato, dehydration was evidently reflected drought-responsive genes related to the alteration of the chloroplast structure (Chen et al. 2020). Water stress has been known to decrease the capacity of light capture, especially in C3 plants. It was indicated that photosystem II (PSII) was more disturbed by water stress than photosystem I (PSI), and it means that *LHCB* gene seemed to be more sensitive than *LHCA*. Similar results in rice seedling, water-stress obviously decreased the light absorption by antennae and also reduced the rates of electron transport (Dalal and Tripathy 2018). In Arabidopsis, the absence of one of the *LHCA1/LHCA4* and *LHCA3/LHCA2* dimers caused structural changes that unable to be replaced by other *LHCA* proteins. Loss of functions of *LHCB1* and *LHCB2* will lead to reduce light absorption, while loss of function of *LHCB5* and *LHCB6* will induce declining the efficiency of energy transfer from *LHCB* to PSII reaction center (Sasi et al. 2018).

Drought stress known to cause disruption of functions of structural and carrier proteins and also decrease the excitation rate of electrons at the reaction center of photosystems. Changes in expression levels of structural protein genes in the reaction center seemed not directly to cause loss of its functions (Wada et al. 2019). Our result showed that among genes related to photosystem-electron transfer chain there was one gene encodes enzyme 1.18.1.2 (*FNR2*) that disrupted by water stress (Fig. 3), and has been known to cause immediate obstruction at the end of electron transfer process. The disruption of FNR2 enzyme will inhibit NADPH production, which plays an important role as the source of energy in carbon dioxide assimilation of the Calvin cycle, nitrogen-lipid metabolism, chlorophyll biosynthesis and regulation of stromal redox (Li et al. 2017). Water deficiency was also known to downsize the light-harvesting antenna complex to protect from photo-oxidative damage and inducing the reduction of photosynthetic electron transport process. Similar results were reported in rice and potato seedlings (Dalal and Tripathy 2018). Disturbances of drought to photosynthetic electron transport process and damage both PSII and PSI reaction centers had been reported in many plants, including young apple tree (Wang et al. 2018).

Conclusion

This study showed that the addition of 10% PEG induced water deficiency condition in banana plantlets and evidently affected not only chlorophyll biosynthesis, but also altered the biochemical reactions in Calvin cycle, disturbed the light-harvesting capacity and disrupted the electron transfer process in photosystem chains. Water deficiency made impacts to the expressions of 52 genes related to photosynthesis process of *M. acuminata* Colla cv 'Barangan Merah' plantlets. The 52 genes were allocated in chlorophyll biosynthesis (4 genes), in Calvin cycle (9 genes), in light harvesting complex (15 genes), and in electron transfer-photosystem chain (21 genes). Based on relative gene expression values of the 52 genes showed that a total of 45 genes were down-regulated, and 8 genes were up-regulated. Results of transcriptomic analysis in this study will be useful for developing drought-resistant banana plants in the future.

Author's Contributions

SNW: Conceptualization, research supervision, writing of original draft manuscript; SS & SD: Methodology, formal analysis, research investigation; EM & HN: Visualization, review and editing of manuscript; FSI: Data curation, formal analysis; DSD: Critically review and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Disclosure

The authors declare that they have no conflicts of interest in the research.

Acknowledgement

This research was supported by: the Master Degree to Doctoral Scholarship Program for Excellence Undergraduate (PMDSU), Directorate General of Higher Education, Ministry of National Education, Indonesia (2019-2020); and the Program of Research, Community Service, and Innovation (2020-2021), School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, Indonesia.

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