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Studying the Transcriptome Profiling of Banana Plantlets Exposed to Water Stress and the Alteration in Their Major Bioprocesses

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Abstract

Water shortage has been one of the major problems that limit the production yield of banana. The molecular mechanisms by which banana plants thrive in drought are not completely understood. This study aimed to reveal the major bioprocesses affected by water shortage in banana plantlet using a transcriptomic analysis approach. *In vitro* shoot cultures of banana (*Musa acuminata*) cv Barangan Merah were established on water stress treatments with the addition of polyethylene glycol (PEG) in the culture medium. Banana plantlets generated from water stressed shoots from the control (BK), low 2.5% PEG), medium (7.5% PEG) and high (10% PEG) levels were utilized as the resource for total RNA samples. Four cDNA libraries were constructed and sequenced using the Illumina MiSeq™ 2000 platform. Transcriptome data analysis was conducted. From a pool of four transcriptome libraries, each consisting of about 3,500,000 paired-end raw reads, 147,811 contigs were assembled, from which 129,701 contigs were annotated with SwissProt reference and *Musa acuminata* gene model. A total of 101,406 high quality and non-redundant contigs were obtained for differential expressed genes (DEG) analyses. Gene ontology was performed using DAVID, followed by pathway prediction using KEGG. Statistical analysis identified 1,744 genes as DEGs under PEG treatment in which 1,046 genes (67%) of them were mapped to the reference genomes. These DEGs were distributed in 26 functional clusters. There were eight major biological processes that were highly affected by the drought stress in banana, including photosynthesis, cellular redox balance, cellular components stability, cellular energy preservation, metal ion homeostasis, hormonal-activated signaling pathways, organ development, and production of transcription factors (TFs). There 47 genes encoded for TFs were identified including five families that are typical for stress-responsive genes families (MYB, WRKY, bZIP, ABF, DRE), also auxin- and ethylene-activated TFs and WUSCHEL-related homeobox. Fifteen DEGs were selected for qRT-PCR validation and their expression results were confirmed.

Keywords: Drought stress, *Musa acuminata*, shoot cultures, transcriptome profile.

1. Introduction

Bananas (*Musa* spp.) are famously known as a commercial crop in tropical and subtropical countries, including Indonesia. For optimal growth, banana plants require a warm and humid climate. The existing commercial cultivars of banana are mostly parthenocarpy and generated from hybridization between one or two of the major diploid ancestors, *Musa acuminata* (A-genome) and *Musa balbisiana* (B-genome) that produce the three triploid hybrids, either with AAA, AAB, or ABB genome constitution (Nayar, 2010 and Davey *et al*., 2013). Naturally, banana plants are exposed to suboptimal field conditions throughout its life cycle. Drought is one of major abiotic stresses that is affecting banana production worldwide (Santos *et al.,* 2018) because insufficiency of water supply will reduce the growth of banana plants and cause a big loss of production yield of banana fruits (Nansamba *et al.,* 2020).

Drought refers to the condition of water deficit when water levels drop below a certain threshold. The water deficit condition occurs either when the water supply becomes limited or when the transpiration rate becomes intense. The effects of plant water stress vary between the plant species. The most common early symptom of plant water stress is when the leaves wither and dry. Recognition of water-stress symptoms can be critical to maintaining the growth of a crop (Bhattacharjee and Saha, 2014). When the plant encounters water stress, the osmotic pressure inside leaf cells decreases and causes the leaves to wilt. To get through with the drought, tolerant plants initiate defense strategies against water deficit that can be expressed at the phenotypic levels and the molecular levels (Ilyas *et al*, 2021). In response to water stress, changes occurred in the cellular level, including changes in metabolic pathway directions, changes in the nutrient and

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ion uptakes, synthesis of new proteins and modulation of free radical generation (Omprakash *et al.*, 2017).

Drought has been known to cause morphological, physiological, biochemical, and molecular changes in banana. Water stress evidently changed morphological and physiological activity, that affected the growth and productivity of plants. Morphological characteristics of banana plants, such as the numbers of leaves, shape and color appearance of leaves, reduction of the greenness of leaves, and plant heights were significantly changed under drought conditions (Uwimana *et al*, 2021). It was found that the lack of sufficient water also affected the relative water content (RWC), soluble protein content, total chlorophyll, and photosynthetic pigment contents of various banana genotypes. Water deficiency also gave an impact on increasing the biosynthesis of epicuticular wax, proline, and free amino acid in banana plants (Surendar *et al.*, 2013). Nevertheless, studies showed that drought tolerance in banana plants was genotype-dependent. It was indicated that different cultivars of banana are varied in their tolerance levels to drought conditions. Among the three-triploid banana hybrids, i.e. the AAA, AAB, or ABB genomes, the cultivars with ABB genome constitution are known to be more tolerant to drought (Wang *et al*., 2020).

Understanding the molecular basis of how banana plants respond to water stress is the main key to developing drought tolerant banana plants in the future. Molecular studies showed that banana plants are capable of super-expressing genes related to resistance and defense response to abiotic stresses, including drought and salinity stresses (Hu *et al*., 2017). The molecular regulation mechanisms of banana in response to various abiotic stresses have been extensively studied using the next generation sequencing (NGS) technology. The NGS technology is known to be very useful in generating large amounts of expressed sequence tags (EST) which is adequate to cover the transcriptome of bananas (Lowe *et al.*, 2017). A draft genome of the wild *M. balbisiana* diploid variety, the 'Pisang Klutuk Wulung' ('PKW', B-genome) was successfully sequenced and assembled (Davey *et al*., 2013). The draft genome was used in comparative transcriptomics and genomics studies of interspecific triploid and tetraploid banana hybrids. The computational prediction, identification, and expression profiling of microRNAs had been used to identify drought-responsive miRNAs in the EST library of drought-stressed banana. The miRNAs are post-transcriptional gene regulators and implied to regulate gene expression during drought response (Muthusamy *et al.*, 2014).

Based on NGS-technology, Backiyarani *et al.* (2015) accomplished the transcriptome analysis of banana (*Musa balbisiana*). The transcriptome sequencing was performed using the Ion Torrent platform and generated about 4.5 million paired-end reads. The RNA sequencing results provided transcriptomic information on the *Musa* sp. 'B' genome and led to the development of trait-specific markers and also the discovery of new genes and regulatory sequences that are involved in resistance mechanisms. Likewise, transcriptome analysis had been employed to identify and compare drought stress-responsive long non-coding RNAs (lncRNAs) from drought-tolerant and -susceptible banana (*Musa* spp) cultivars. The LncRNAs are known to be responsive to biotic and abiotic stresses and regulate genes to respond (Muthusamy *et al.*, 2015). Muthusamy *et al.* (2016) also discovered the transcriptomic changes of tolerant and sensitive banana cultivars exposed to drought stress. The differences in profiles were determined between drought-tolerant cv Saba (ABB genome) and the drought-susceptible cv. 'Grand Naine' (AAA genome) in tolerance mechanism to water deficiency. It was verified that comparative physiological and transcriptomic analyses were applicable to reveal the integrated insight into abiotic stress tolerance mechanisms in banana. Based on phenotypic and physiological analyses, it was confirmed that the ABB genotype banana had the strongest tolerance to abiotic stresses compare to the AAA and AAB genotypes (Wang *et al*., 2020).

Studies showed comparative transcriptomic analyses had confidently improved our understanding of the molecular mechanisms of defense occurred in *Musa* spp. when plants under drought stress. Transcriptome sequencing technologies successfully generate and provide a framework dataset that is needed to be further analyzed for transcriptome mapping, determining metabolic pathways, clarifying gene expression patterns, and identifying new genes (Rani and Sharm, 2017). Basically, the information contents of transcriptome data are recorded as a snapshot at the moment taken, hence the information contents will be very specific and will not be the same in different tissues or conditions, or at different times. Nevertheless, transcriptome data give us information on how genes are regulated and reveal details of biological processes occurring in cells, tissues, or organ parts of an organism at a certain time and in a specific condition; therefore, drought responsive gene expression profiles of banana remain unexplored thoroughly (Hu *et al*., 2017). Accordingly, it was emphasized that there is a need for more studies using the transcriptome sequencing technology to discover the molecular mechanisms of banana in response to water stress. A comprehensive transcriptome data will further enhance our understanding of the mechanisms regulating drought tolerance in banana and determine the play roles of expressed genes in stress regulation and post-translational mechanisms (Mattos-Moreira *et al*., 2018). This study aimed to unravel the mechanism of banana plantlets in response to water stress condition through morphological observation and transcriptional analysis. A reference transcriptome dataset was generated from cDNA libraries of banana plantlets, *Musa acuminata*, Colla cv Barangan Merah (AAA genome), which is known as a drought-susceptible cultivar (Sebayang *et al*., 2018). In this study, transcriptomic changes of the water stressed plantlets of Barangan Merah banana cultivar were analyzed to discover the gene expression profiles related to the major biological processes.

2. Materials and Methods

2.1. Plant Materials and Treatments

In vitro shoot cultures of banana (*M. acuminata*) cv Barangan Merah used in this study were obtained from SEAMEO BIOTROP, Bogor, Indonesia. Banana shoots were cultured on MS (Murashige and Skoog, 1962) basal medium (PhytoTech Lab. USA) supplemented 3 % (w/v) sucrose and 0.8 % (w/v) agar (Swallow Globe, Indonesia)

to solidify. Emerged axillary shoots were sub-cultured every four weeks and used as explants for water stress treatments. Polyethylene glycol (HiMedia Laboratories, India) was added to the culture medium to induce water deficiency. Three different PEG concentration levels used in experiments were 2.5% (coded as BP2), 7.5% (coded as BP7), and 10 % (coded as BP10), and those were equivalent to osmotic potential (OP) at -0.19, - 0.93, and - 1.48 bars (Michel and Kaufmann, 1973). Shoots grown on culture medium without the addition of PEG were used as the control treatment (coded as BK). After a four-week period of culturing, shoots were rooted and regenerated into plantlets on MS medium supplemented with 5 µM 6-Benzyl-aminopurine (Sigma-Aldrich, Singapore). All banana in vitro cultures were maintained in a culture room at 22 ± 2˚C under continuously-lighting at 2,000- 3,000 lux (Bharati *et al.*, 2018). Six replicates were made for each treatment. Three replicates of each sample were prepared for RNA extractions and three other replicates were used for subculturing and further experimental analyzing.

2.2. Morphological Observations

Morphological observations were carried out to evaluate the effects of water stress on the growth and morphological changes of banana plantlets. The physical appearances of the four groups of banana plantlets (BK, BP2, BP7, and BP10) were compared. The shoot lengths of plantlets (height in mm), number of leaves, and shoot multiplication rates were used as the growth parameters and measured after a four-week period of PEG exposures. Changes in the appearance of leaves, reduction of the greenness of foliage, and the evidence of necrotic, chlorosis, or browning tissues of plantlets were also monitored. For morphological observation, six replicates were made for each treatment, and the statistical analysis was determined by One-way ANOVA and Tukey test using the SPSS software version 23.0.

2.3. RNA Extraction

For transcriptome sequencing, tissue samples of the four groups of banana cv Barangan Merah plantlets (BK, BP2, BP7, and BP10) were collected and prepared for the RNA isolation. The total RNA of each group of treatment was extracted separately using the CTAB-LiCl protocol (Song *et al.*, 2011). Frozen tissues of banana plantlets were ground into fine power in liquid nitrogen using a pre-cooled mortar. The fine powder samples were then submerged into 0.1% (v/v) cethyl trimethyl ammonium bromide buffer (CTAB Merck, Singapore), and followed by two times extractions with chloroform. The RNAs were precipitated using lithium chloride (Merck, Singapore), and then extracted using chloroform again. The least RNAs were precipitated with ethanol, and the pellets were resuspended in 20-100 µL diethyl pyrocarbonate (DEPC) treated water (ThermoFisher Scientific, USA). The integrity of RNAs was assessed with the Agilent 6000 RNA Nano Chip Kit on 2100 Bioanalyzer (Agilent Technologies), following the procedure described by Diningrat *et al.* (2015).

2.4. cDNA Library Construction and Transcriptome Sequencing

Two-paired-end cDNA libraries were developed according to the protocol of Two-Paired-End Sample Preparation Kit (Illumina Inc., USA). The four cDNA libraries were constructed and sequenced using the Illumina MiSeq^{TM} 2000 platform (Diningrat *et al.*, 2015). Only one transcriptome dataset was presented for each treatment group. Transcriptome raw data were generated from four cDNA libraries of banana plantlets, and coded as BP2, BP7, BP10, and BK as the control.

2.5. Transcriptome Data Analysis

Banana transcriptome data analysis was conducted through several steps as illustrated in the bioinformatics analysis flow chart (Figure 1). Quality assessment of all raw FASTQ reads was conducted using FastQC version 0.11.8 prior to *de novo* transcriptome assembly. *De novo* transcriptome assembly was performed using Trinity assembler package version 2.8.4 (commission no. 4539805) with a k-mer size of 25 and both sequencing adapter and bases with low quality scores were removed with Trimmomatic version 0.36 (Haas *et al.*, 2013). Transrate version 1.0.3 was used to assess the quality and filter the assembled contigs, retaining only contigs that have Transrate contig quality scores greater than or equal to 0.04776 (Smith-Unna *et al*., 2016).

Contigs that passed the filtering step by Transrate were used as input for TransDecoder (version 5.5.0) for the detection of the putative open reading frame (ORF). Contigs were then annotated using Trinotate version 3.1.1 with all the contigs passing Transrate filtering step and ORFs predicted by TransDecoder as inputs. Contigs were annotated against Swiss-Prot database (The Uniprot Consortium, 2019), Pfam 32.0 (El-Gebali *et al.*, 2019) and *Musa acuminata* genome assembly and annotation-version (Martin *et al.* 2016). Contigs expression level for each treatment group was determined using the align_and_estimate_abundance.pl script as part of the Trinity package. Quality trimmed reads from each treatment were mapped using Bowtie2 version 2.3.5.1 (Langmead and Salzberg, 2012) read counts were estimated using RSEM version 1.3.2 (Li and Dewey, 2011).

Differential expression analysis was done in RStudio version 1.2.1335 (RStudio Team, 2018) using DESeq2 package (Love *et al.*, 2014). To reduce the probability of false positive error, we removed any contigs with an expression level less than the expression level at the 85th percentile across all contigs. For gene expression analysis, the number of expressed tags was calculated and normalized to the number of transcripts per million (TPM) tags. Each unigene was grouped based on the ratio of TPM to that of the control data. Unigenes were analyzed with a cut-off ratio of log10 > 2 to obtain a list of unigenes with multiples of more than 100 times compared to those of the control group (p-value $\leq$ 0.001). The differentially expressed tags were used for further mapping and annotation. To visually represent the similarities and differences of functional gene expressions among the three groups of treatments (BP2, BP7 and BP10), the Venn diagrams were made up and generated using a web-based tool by PSB Ugent (http://bioinformatics.psb.ugent.be/webtools/Venn/).

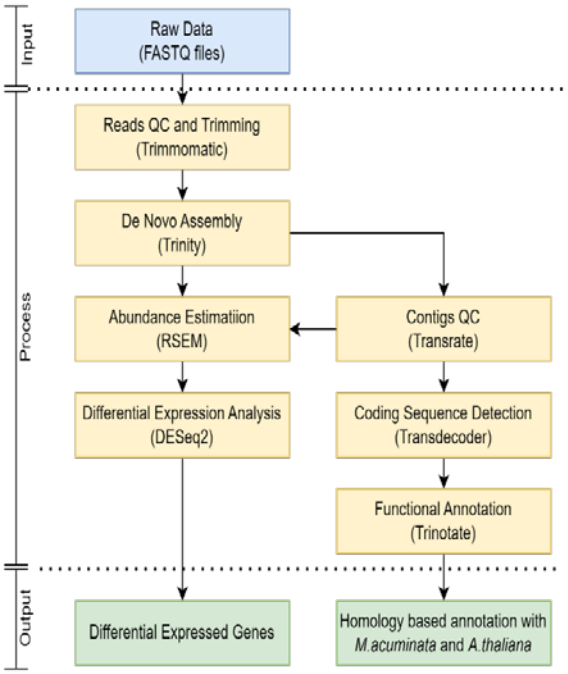


Figure 1. Bioinformatics analysis flow chart for transcriptome profiling of banana plantlets under drought stress.

2.6. Gene Ontology (GO) Enrichment Analysis

Further analysis of GO enrichment was accomplished only for the high concentration treatment, the 10% PEG (BP10 plantlet) as a representative of other treatments. The GO enrichment analysis of the differentially expressed genes was completed using the Database for Annotation, Visualization, and Integrated Discover (DAVID) at http://david.abcc.ncifcrf. gov/. The pathway analysis was performed based on *Arabidopsis thaliana* (TAIR) annotation and the Kyoto Encyclopedia of Genes by and Genomes (KEGG) http://www.genome.jp/kegg/.

2.7. Validation of Selected Gene Expressions by qRT-PCR Assay

To confirm the expressions of selected annotated DEGs, an independent experiment was carried out to replicate a similar experiment with previous experiments, except that for validation of gene expressions, the total RNA samples were extracted from banana cv Barangan Merah plantlets of the BK (control treatment without PEG) and the BP10 (with 10% PEG treatment) only. The total RNA samples were isolated from plantlets of the BK and the BP10 after four weeks of the PEG treatment. The RNA extractions were done using CTAB-LiCl method as described previously (Song *et al*., 2011). Three biological replicates were used for the validation. The quality and quantity of RNA were measured using Nanodrop™ Lite Spectrophotometer (Thermo Scientific, USA) and electrophoresis was confirmed in 1.5 % agarose (Kusdianti *et al.*, 2016). The cDNA constructions were carried out with the GoScript™ Reverse Transcription System according to manufacture manual (Promega, USA). The cDNA synthesis reaction was done by incubation for 5 minutes at 25 °C, followed by incubation for 1 h at 42 °C and deactivated of reverse transcriptase for 15 minutes at 70 °C. The quantity of total RNA measured using nanodrops showed an average of 273.3 ng/µl (Amalia *et al.*, 2016). Construction of cDNA with reverse transcriptase enzyme generated banana cDNA that can be used for qRT-PCR analysis.

Fifteen genes selected from annotated DEGs (Supplementary Table S.1) were subjected to quantitative real-time PCR (qRT-PCR) assay. Primer pairs were designed using the Primer3Plus package following the procedure described at https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi. All designed primers were checked with the Primer Blaster (https://banana-genome-hub.southgeen.fr/primer blaster) and compared to the banana genome available at CIRAD to ensure the primers would hit with the *M. acuminata* (AAA genome). The primers had a specification of 18-25 bases length (Macrogen, Singapore), 40-60% GC percent, the temperature of melting (Tm) between 55-65^{o}C, and no self-complementary or dimer. Analysis with Primer Blaster software also showed the same amount of amplification with Primer3Plus software, thus the primers could amplify the target genes and used for gene expression analysis.

Quantitative Real-Time PCR (qRT-PCR) analyses were performed using QuantStudio 1 (Termo Scientific, USA). The qRT-PCR was performed using GoTaq® qPCR Master Mix according to the manufacturer manual (Promega, USA). The PCR reaction procedure (Amalia *et al*., 2016) was started with pre-denaturation at 95 °C for 15 minutes, followed by 40 cycles of polymerization (15 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C). Three technical replicates were applied in the qRT-PCR assay. Relative fold expression values were normalized using *MaACT* (actin) and *MaBT* (betatubulin) as the housekeeping genes and calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Statistical analyses are used to estimate the significance of statistical program and presented graphically (Hu *et al*., 2017).

3. Results

3.1. Morphological Evaluations

Visible morphological changes occurred in plantlets after four weeks of PEG exposure. Morphologically, plantlets of the BP2, BP7 and BP10 were very different in their appearance compared to plantlets of the control group (BK). Remarkable signs were noticed as the reduction in the greenness of foliage, changes in shoot colors, reduced size of leaves, and fewer leaves turgidity in plantlets (Figure 2.A-D). Some leaves of plantlets turned yellowish after four weeks cultured on a water shortage condition, particularly on medium with 10% PEG. Morphological changes might be related to the expression of specific genes related to the major biological process that occurred in plantlets as a response to water shortage. The lack of water may further reduce most downstream processes that require water, primarily photosynthesis but also increase the effectiveness of energy formation and uses- the details will be discussed later. It seemed to be corresponding to the changes in transcriptome profiles.

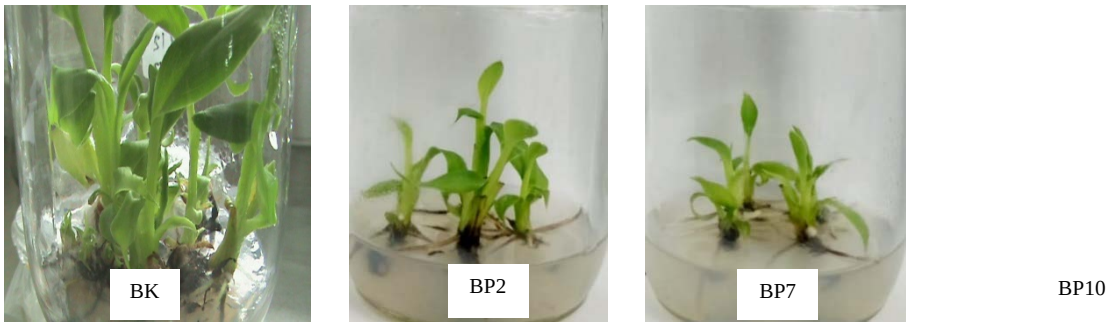


Figure 2. (A) Banana plantlets of the control (BK), (B) treatments with PEG 2.5% (BP2), (C) PEG 7.5% (BP7), and (D) PEG 10% (BP10) after four weeks of PEG treatments which were used as the sources of RNA samples for generating the transcriptome data.

The evaluation showed changes in shoot lengths, numbers of leaves and shoot multiplication rates of banana plantlets after four weeks of exposure to PEG in MS treatments (Figure 3 A-C). Compared to those of the control treatment (BK), plantlets of the BP2, BP7 and BP10 obviously decreased their growth. The averages of heights (shoot length in mm) of the BP2, BP7 and BP10 plantlets were 38 mm, 26 mm and 19 mm which were very much lower compared to those of the control ones (65 mm). The water deficiency also greatly reduced the leaf numbers of plantlets. Drought stressed plantlets had lower numbers of leaves on averages of 3.8 (BP2), 2.5 (BP7) and 2.3 (BP10) whereas the control plantlets evenly had higher numbers of enlarged leaves (6.3 per plantlet). Other than that, the lack of sufficient water seemed to suppress the growth ability of axillary buds causing shoot multiplication rates were excessively declined, especially at 10% PEG (BP10).

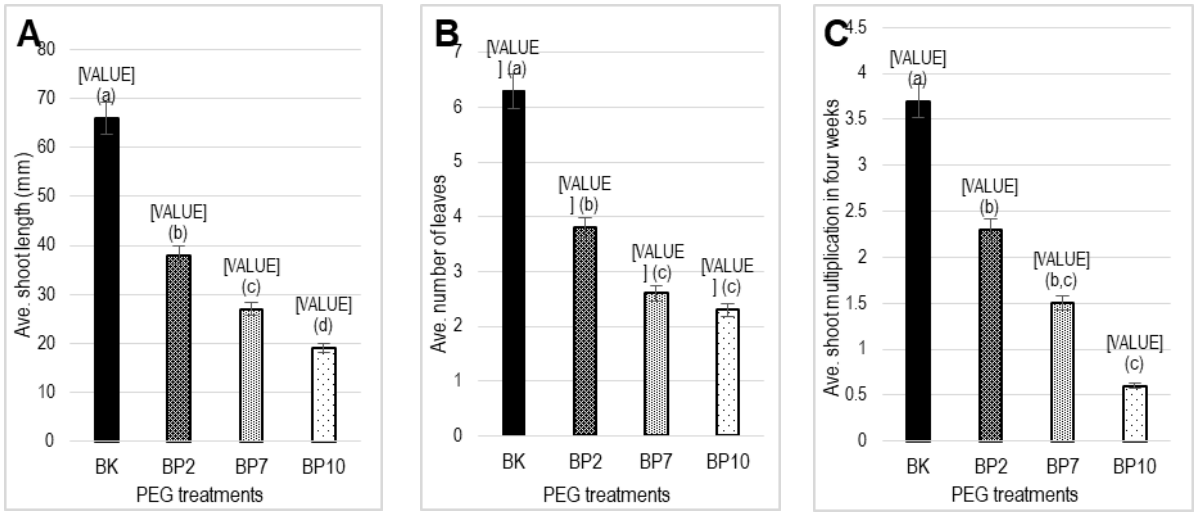


Figure 3. (A) Average of shoot lengths (mm), (B) average of leaf numbers, and (C) average of shoot multiplication rates of banana plantlets: BK, BP2, BP7 and BP10 four weeks after exposure to PEG in MS treatments. The number at top of each bar represents the mean value with error bars at p = 0.05. Values followed by the same letters are not significantly different at 0.05 level as determined by One-way ANOVA and Tukey test.

3.2. Transcriptomic Analyses

A transcriptome dataset was generated from four cDNA libraries of banana plantlet groups that were exposed to water stress by the addition of PEG (BP2; BP7, BP10) and the control treatment (BK). All RNA-seq data had been submitted and registered with the BioProject database NCBI (BioProject ID PRJNA970186). In these four transcriptome libraries, each one consisting of ~3,500,000 paired-end raw reads, a total of 147,811 contigs were assembled, from which 129,701 contigs were annotated with SwissProt reference and *Musa acuminata* gene model. After the redundant and low quality contigs were filtered out, a total of 101.406 high quality and non-redundant contigs were obtained for further analyses. Contigs length ranged from 200 to 1800 bp with N50 value of 998. Transcriptome assembly statistics are listed in Table 1.

Table 1. Transcriptome statistics of the assembled contigs of banana plantlets of the low quality- and redundant- contigs were omitted with the TransRate tool.

Parameters	Values
Numbers of bases	104,118,407
Numbers of contigs	147,811
Smallest contig size (in bases)	199
Largest contig size (in bases)	7,393
Percentage of fragments mapped (%)	91.8
Percentage of good mapping (%)	77.3
Percentage of contigs uncovered (%)	17.5

3.3. Differentially expressed genes (DEGs) Analysis

Genes with very low expression levels were excluded from the 101,406 high-quality contigs and resulting in

60,637 genes being used as input in the Differential Expressed Gene (DEG) analysis. Further filtered-out with the threshold of the adjusted p-value of 0.05 resulted from a total of 8,444 contigs were obtained and used in pairwise analysis. All DEGs were divided into three groups based on its PEG treatments. The total numbers of 1,564 genes, 1,556 genes, and 1,744 genes were finally identified as differentially expressed (DEGs) following the treatments of 2.5% PEG (BP2), 7.5% PEG (BP7), and 10% PEG (BP10), respectively. In Venn diagrams (Figure 4), gene numbers in a circle showed the relationships of genes among groups. The numbers in overlapped circles exhibited similar expressions, while numbers in separate circles indicated the number of genes which did not take a part in the same trait. The three groups were BP2, BP7 and BP10 groups and showed a similar ratio of two-thirds (2/3) of up-regulated and down-regulated genes. In BP2 group there were 631 genes (40.3%) up-regulated, and 933 genes (59.7%) were down-regulated. In BP7 group, 658 genes (42.2%) were up-regulated, and 898 genes (57.8%) were down regulated, while in BP10 group, 762 genes (43.7%) were up-regulated, and 982 genes (56.3%) were down regulated (Figure 4, Table 3).

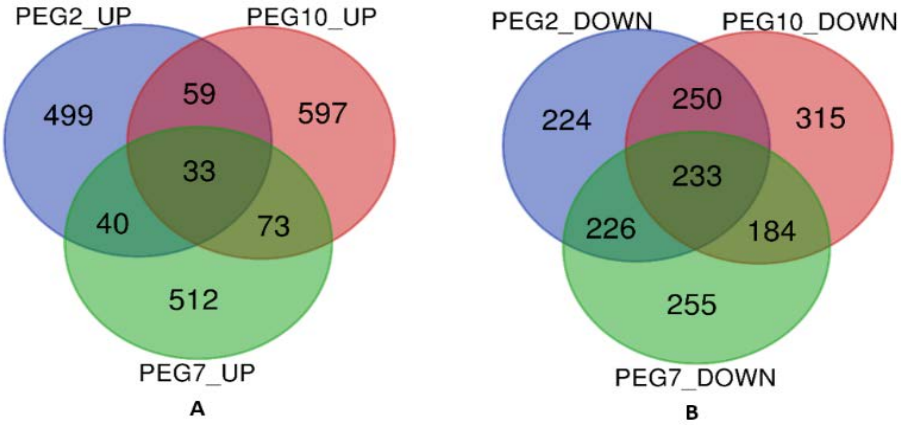


Figure 4. The Venn diagrams of the total gene counts of DEGs: (A) Upregulated genes and (B) Downregulated genes, in 2.5% PEG, 7.5%PEG, and 10% PEG.

A total of 25 annotated genes were consistently upregulated in all PEG exposed plantlets (BP2, BP7, and BP10) which consisted of genes responsible for the processes of defense to abiotic and biotic stresses, the metabolic process of the antioxidant glutathione, abscisic acid-activated signaling pathway, cell redox homeostasis, photorespiration, DNA repair and protein modifications (Table 2). The results of the DEG analysis also indicated genes that were exclusively expressed in each exposure to PEG treatment, with the biggest portion of the genes occurring in the highest PEG concentration (10% PEG). Using homology comparison with two genome references, the previously published *Musa acuminata* cv *Pahang* genome model and *Arabidopsis thaliana*, we were able to assign the functions of at least two-third of the up-regulated and down-regulated genes in each treatment group.

On the other hand, 233 genes (94 of them were annotated) were consistently down regulated in all tested concentrations of PEG (Figure 4) with the majority involved in the formation of photosynthetic machinery such as chloroplast membrane components, photosystem I and II, that play role in light harvesting during photosynthesis. In addition to that, genes related to the process of cellular ion homeostasis, amino acid transmembrane transport, ethylene- and auxin- activated pathways, fatty acid biosynthesis, cell wall biogenesis, plant organ morphogenesis, oxidoreductase activity, phenylpropanoid metabolic process, and response to abiotic and biotic stimulus- were also appeared to be consistently down-regulated in all treatments (Supplementary Table S.2).

Out of 762 up-regulated genes, 100 genes were selected as genes with the highest expression levels enhanced by at least a thousand times. Among the genes are early nodule-like protein, L-ascorbate peroxidase, acidic endochitinase, protein disulfide isomerase-like, superoxide dismutase, prolyl 4-hydroxylase, nucleolin 2, gibberellin-regulated protein, tubulin alpha-1 chain, flavonoid 3'-monooxygenase, small nuclear ribonucleoprotein G, and stearoyl-[acyl-carrier-protein] 9-desaturase (Supplementary Table S.3). Interestingly, the top 100 up-regulated genes represented quite a large variety in function. Based on their functions, these genes can be classified into eight major roles i.e., (1) photosynthesis, (2) cellular redox balance; (3) cellular components stability; (4) cellular energy preservation; (5) metal ion homeostasis; (6) hormonal-activated signaling pathways; (7) production of transcription factors; and (8) organ development.

Table 2. A total of 25 annotated genes were consistently upregulated in all PEG exposed plantlets (BP2, BP7, and BP10).

Contigs ID	Biological process	Cellular components	Molecular function
MaB_DN812_c1_g1	DNA repair; response to salt stress	cytosolic small ribosomal subunit	damaged DNA binding
MaB_DN94_c0_g2	Protein sumoylation	cytoplasm; nucleus	protein tag ubiquitin-like protein
MaB_DN1392_c1_g1	Protein refolding root development	chloroplast; endoplasmic reticulum	peptidyl-prolyl cis-trans isomerase
MaB_DN75017_c0_g1	Polar nudeus fusion; rRNA pseudo	box H/ACA snoRNP complex; box H/ACA telomerase RNP	box H/ACA snoRNA binding telomerase RNA

	uridine synthesis	comp.	binding
MaB_DN180_c0_g3	Spliceosomal snRNP assembly	U12-type spliceosomal complex	mRNA binding
MaB_DNS2396_co_g1	.	endoplasmic reticulum membrane	.
MaB_DN6130_c1_g4	.	endosome; plasma membrane;	peptidyl-prolyl cis-trans isomerase
MaB_DN16107_c0_g1	.	plasma membrane	.
MaB_DN999_c1 g1	.	eukaryotic translation initiation	mRNA binding; ribosome binding;
MaB_DN10_c1_g1	.	cytoplasm, nudeus	.
MaB_DN8775_c3_g1	Defense response to bacterium	plasma membrane	magnesium-dependent protein
MaB_DN767_c1_g2	.	nucleosome; nucleus	protein heterodimerization activity
MaB_DN31726_c0_g1	Cytokinin metabolic process extracellular space	extracellular space	cytokinin dehydrogenase activity;
MaB_DN3960_c1_g1	Glutathione metabolic process,GO:0009407 toxin catabolic cytoplasm,GO:0005829	cytoplasm,GO:0005829 cytosol	Glutathione transferase activity
MaB DN3032_c1_g1	Hydrogen peroxide catabolic	cell wall; plasmodesma; vacuole cytoplasm	heme binding; metal ion binding;
MaB_DN39392_c1_g1	.	cytoplasm	metal ion binding; superoxide
MaB_DN17680_c0_g1	Intracellular protein transport;	chloroplast, clathrin coat of trans-	clathrin light chain binding; structural
MaB_DN3027_c0_g1	Chromatin-mediated maintenance	transcription elongation factor	metal ion binding; RNA polymerase
MaB DN6388_c0_g1	Cell cycle arrest; cellular response to DNA damage stimulus	cytoplasmic ribonucleoprotein granule; ubiquitin ligase complex	kinase binding; metal ion binding; protein heterodimerization activity
MaB_DN2680_c0_g2	GO:0005412_translation	cell wall; cytosolic small ribosomal	structural constituent of ribosome
MaB DN16624_c0_g1	Abscisic acid-activated signaling	cytosol; nucleus	magnesium-dependent protein serine/threonine phosphatase
MaB_DN138_c0_g2	Defense response	cell wall; extracellular region	.
MaB DN7483 c0 g1	Glutathione metabolic process;	chloroplast: cytoplasm; cytosol	Glutathione binding; glutathione
MaB DN20701_c0_g1	Cell redox homeostasis	endoplasmic reticulum lumen	protein disulfide isomerase activity
MaB_DN47361_c0_g2	Photorespiration	mitochondrial respiratory chain	NADH dehydrogenase (ubiquinone)

Table 3. Summary of the contig annotation and differentially expressed genes (DEGs) analysis. Numbers presented in each column are total gene counts resulting from each step described in the column title.

Sample	Assembled contigs	Total annotated	Numbers of DEGs	Upregulated	Downregulated
BK (Control)	147,811	129,701	-	-	-
BP2 (2.5% PEG)			1,564	631 (40.3%)	933 (59.7%)
BP7 (7.5% PEG)			1,556	658 (42.2%)	898 (57.8%)
BP10 (10% PEG)			1,744	762 (43.7%)	982 (56.3%)

3.4. Functional Cluster Analysis of DEGs

Using *A. thaliana* genome model as a reference, all differentially expressed genes were clustered in 26 functional groups with the largest being constituted by genes playing roles in transmembrane-transports (1,307 genes), photosynthesis (1,303 genes), transcription factor (TFs) production (1,013 genes), protein phosphorylation (747 genes), cellular secretions and detoxifications (468 genes), and cell redox homeostasis (449 genes). Other functional groups that were also overrepresented by the DEGs are those related to cellular respiration, RNA processing and translation, protein interactions, cell wall re-organization, cellular components and organelles, lipids- and amino acids- biosynthesis and transports, secondary metabolite biosynthesis, and DNA modifications and repairs (Figure 5).

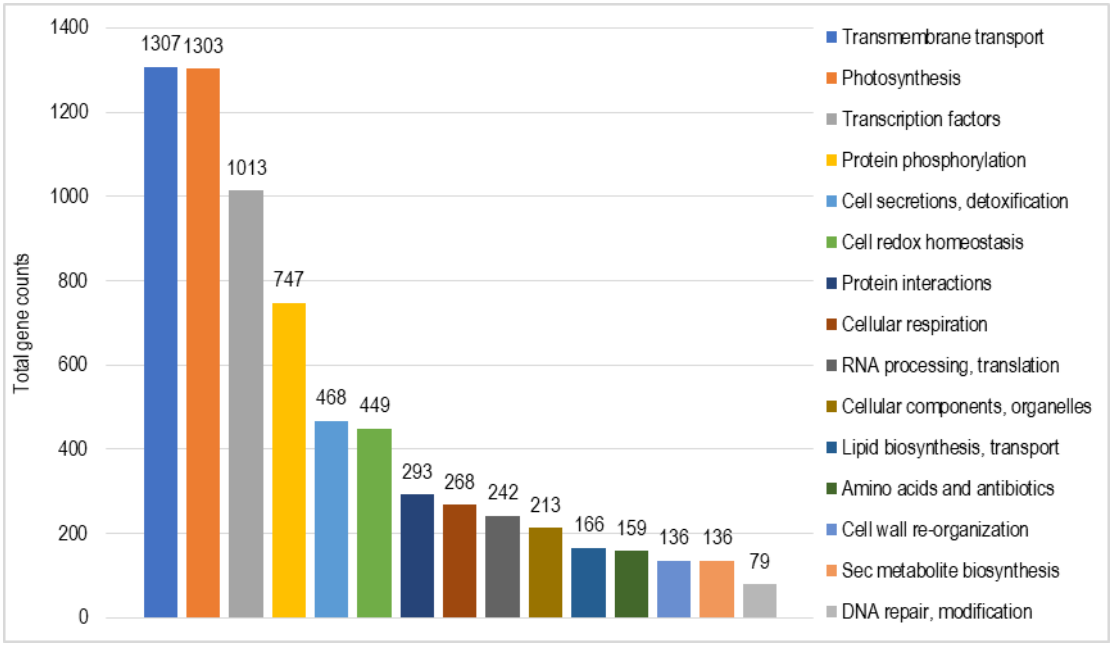
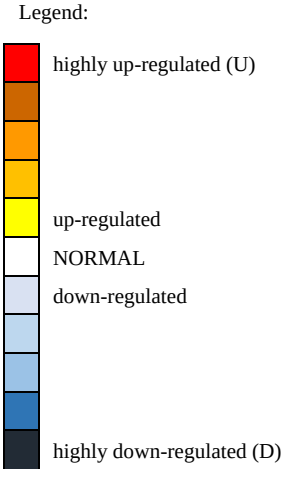


Figure 5. Gene functional cluster analysis of DEGs using DAVID bioinformatics tools.

Further GO enrichment analysis was achieved in detail for the 10% PEG treatment only. Based on DAVID, GO enrichment results of the BP10 plantlets indicated systemic changes that occur upon stress in banana involving large numbers of biological processes, i.e. the cellular process, metabolic process, response to stimulus, developmental process, and multicellular organismal process. The most significantly enriched biological processes are the cellular metabolic process, primary and secondary metabolic process, cellular catabolic process, carbohydrate metabolic process, alcohol catabolic process, the stress response to abiotic stimulus, response to chemical stimulus, cellular process, anatomical structure development, multicellular organismal development, post-embryonic development, and pollen development (Table 4). Through statistical analysis, a total of 1,744 genes were identified as differentially expressed genes (DEGs) under PEG treatment, and 1,046 genes (67%) of them were mapped to the reference genomes (Figure 5). It is important to note that the gene members can be overlapped between clusters, which means a gene can contribute to more than one functional cluster. A big portion of genes were represented in the clusters of transmembrane transport, photosynthesis, transcription factors, and protein phosphorylation. A great majority of genes responsible for photosynthetic apparatus, such as chloroplast thylakoid membrane and plastid envelope, were remarkably downregulated upon the drought stress. The suppression effect appeared to be highly exclusive in chloroplast, but not as much in other cellular structures. Downregulation effects on the macromolecular complex, especially the protein-complex composing the light harvesting complex, photosystem I- and photosystem II- reaction center, were strongly suggested in our study. Unlike other cell components, genes responsible for mitochondria structure were majorly upregulated during water stress. The GO enrichment analysis results showed the 100 highest upregulated and 100 lowest down-regulated genes under 10% PEG treatment (Supplementary Table S.3 and S.4).

Table 4. Highly-enriched GO terms in the transcriptome of the three PEG treatments. The colors represent a level of upregulation and downregulation (see the legend).

Enriched GO term_ general	Enriched GO term_ specified	Stress level					
		PEG 2.5		PEG 7.5		PEG 10	
		U	D	U	D	U	D
Photosynhesis	Light reaction						
	Electron transport chain						
	Light harvesting						
Responses to stimulus	Response to stress						
	Response to abiotic stimulus						
	Response to radiation						
	Response to temperature						
	Response to light stimulus						
	Response to chemical stimulus						
	Response to hypoxia						
Secondary metabolic process	Phenylpropanoid biosynthesis						
	Toxin metabolic process						
Carbohydrate metabolism	Carbohydrate metabolic process						
	Carbohydrate catabolic process						
Cellular process	Cellular metabolic process						
	Cellular biosynthesis process						
	Cell growth						
Developmental process	Anatomical structure development						
	Cell differentiation						
	Organ (fruits, seeds) development						
Multicellular organismal development	Pollen develoment						
	Post-embryonic development						
Establishment of localization	Transport						
	Intracellular transport						
Post-translational protein modification	Cellular protein metabolic process						
	Cellular macromolecule metabolic process						
Cell surface receptor-linked signaling pathway	Transmembrane receptor tyrosine kinase signaling						
Cellular respiration	Respiratoty chain						
	Glycolysis						
	Electron transport chain						
	Electron carrier activity						
Lipid localizaton							
Chemical homeostasis	Cellular cation homeostasis						
	Cellular metal ion homeostasis						
Regulation	Regulation of cellular structures						
	Regulation of primary metabolic						
	Regulation cellular biosynthetis						



3.5. Enriched Transcription Factors Following the exposure with PEG Treatment

The increase of transcription factors (TFs) is the event that leads to the up-regulation of various stress responsive genes. From the list of down-regulated genes of BP10 plantlet sample (with 10% PEG treatment) a total of 47 genes encoding for transcription factor (TF) that belong to at least 26 TF families were identified, including five families that are typical for stress-responsive genes families such as *MYB*, *WRKY*, *bZIP*, *ABF*, *DRE*, auxin- and ethylene-activated TFs and WUSCHEL-related homeobox (WOX). In addition to the major responses, there were other interesting biological processes. The high activation of 13 genes in phenyl-propanoid biosynthesis pathway and 5 genes in the flavonoid biosynthesis pathway, which identity and roles require further studies. Analysis results revealed there were 47 genes in 'PBM' banana plantlets that were likely encoded for Transcription Factors (TFs) which were all down-regulated following the drought treatment (Table 5). At least 26 TF families were identified in this study- with the majority are the members of zinc-finger super family protein (10 genes) followed by the *Ethylene Response Factor (ERF)* (4 genes), *MYB* and *MYB*-like TF family protein (3 genes), homeobox-leucine zipper family protein (bZIP) (3 genes), and *WRKY- DNA binding protein* (3 genes). Other TF families are listed in Table 5.

Table 5. Enriched transcription factors of banana plantlets after a four-week exposure to the 10% PEG treatment**.**

M. acuminata cv Barangan_ID	Transcription factors (TFs)
MaB_DN44306_c0_g1	GATA type zinc finger transcription factor family protein (GNC)
MaB_DN74488_c0_g1	Myb domain protein 4 (MYB4)
MaB_DN98776_c0_g1	Basic helix-loop-helix DNA-binding superfamily protein (BIM1)
MaB_DN4733_c0_g1	B-box type zinc finger protein with CCT domain-containing protein
MaB_DN10455_c0_g1	Myb domain protein 16 (MYB16)
MaB_DN41764_c0_g1	Homeobox-leucine zipper family protein/lipid-binding START domain-containing protein (ANL2)
MaB_DN41811_c0_g1	Indole-3-acetic acid 7(IAA7)
MaB_DN76283_c0_g1	Transducin/WD40 repeat-like superfamily protein (SAP)
MaB_DN19921_c1_g1	WRKY DNA-binding protein 34(WRKY34)
MaB_DN72367_c0_g1	GRAS family transcription factor (HAM1)
MaB_DN48622_c0_g1	Basic helix-loop-helix DNA-binding superfamily protein (AT2G41130)
MaB_DN73622_c0_g1	Sigma factor E(SIGE)
MaB_DN17935_c0_g1	GOLDEN2-like 2 (GLK2)
MaB_DN1504_c0_g2	Myb-like transcription factor family protein (AT3G25790)
MaB_DN11588_c0_g1	C2H2 and C2HC zinc fingers superfamily protein (AT3G49930)
MaB_DN65671_c0_g1	B-box type zinc finger family protein (BBX28)
MaB_DN11415_c0_g1	Dehydration response element B1A(DREB1A)
MaB_DN16139_c0_g1	Zinc finger (CCCH-type) family protein (AT5G58620)
MaB_DN81329_c0_g1	Ethylene response factor 1(ERF1)
MaB_DN64112_c0_g1	WUSCHEL related homeobox 4(WOX4)
MaB_DN76923_c0_g1	Abscisic acid responsive elements-binding factor 3(ABF3)
MaB_DN18967_c0_g1	Indeterminate (ID)-domain 5(IDD5)
MaB_DN1857_c3_g1	Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein (AT1G76580)
MaB_DN7159_c0_g1	WRKY DNA-binding protein 15(WRKY15)
MaB_DN83106_c0_g1	Homeobox protein ATH1(ATH1)
MaB_DN2764_c1_g8	Homeobox-leucine zipper protein 4 (HB-4) / HD-ZIP protein (HAT2)
MaB_DN91310_c0_g1	B-box type zinc finger family protein (BBX31)
MaB_DN40655_c0_g1	Homeodomain-like superfamily protein (MYR2)
MaB_DN313_c0_g2	High mobility group B2(HMGB2)
MaB_DN97433_c0_g1	NAC domain containing protein 73(NAC073)
MaB_DN1150_c0_g3	Integrase-type DNA-binding superfamily protein (AT5G51190)
MaB_DN1416_c1_g3	Integrase-type DNA-binding superfamily protein (AT5G51190)
MaB_DN2208_c0_g1	Subgroup of HMGB (high mobility group B) proteins that have a distinctive DNA-binding motif, the HMG-box domain.

MaB_DN23742_c0_g1	Salt-inducible zinc finger 1;(source: Araport11)
MaB_DN17080_c0_g2	C2H2 zinc finger transcription factor that coordinately activates phytochelatin-synthesis related gene expression
MaB_DN45281_c0_g1	Early auxin-induced (IAA16)
MaB_DN65589_c0_g1	Pathogen-induced transcription factor. Forms protein complexes with itself and with WRKY40.
MaB_DN3939_c0_g4	Homeobox protein similar to GL2
MaB_DN60885_c0_g1	Nuclear factor Y, subunit C13;(source: Araport11)
MaB_DN58665_c0_g1	Member of the RAV family of DNA binding proteins. Contains B3 domain. Recognizes 5'-CACCTG-'3 motif.
MaB_DN38283_c0_g1	TCP family transcription factor. Regulated by miR319. Involved in heterchronic regulation of leaf differentiation.
MaB_DN1713_c0_g2	Basic leucine zipper transcription factor involved in the activation of SA-responsive genes.
MaB_DN11711_c1_g2	Member of the GATA factor family of zinc finger transcription factors.
MaB_DN99807_c0_g1	C2H2-like zinc finger protein
MaB_DN10167_c0_g2	Member of the ERF (ethylene response factor) subfamily B-1 of ERF/AP2 transcription factor family (ATERF-9)
MaB_DN64256_c0_g1	Member of the ERF (ethylene response factor) subfamily B-1 of ERF/AP2 transcription factor family (ATERF-9).
MaB_DN17076_c0_g1	Member of the ERF (ethylene response factor) subfamily B-1 of ERF/AP2 transcription factor family (ATERF-9).

3.6. Validation of the DEG genes by qRT-PCR

The qRT-PCR for randomly selected 15 DEGs was tested to check their expression profiles. This independent experimental validation using qRT-PCR was carried out with cDNA synthesized from shoot parts of the BP10 (10% PEG) plantlets, and the BK was used as the control treatment. The qRT-PCR results showed expressions of selected genes (Figure 6). In several points, the results showed similar trends between qRT-PCR data and RNA-seq data. The discrepancy might be due to the different RNA sources, for validation, the RNA samples for qRT-PCR were collected from shoot parts while for RNA-seq (transcriptome) the RNA samples originated from the whole part of plantlets (shoots and roots).

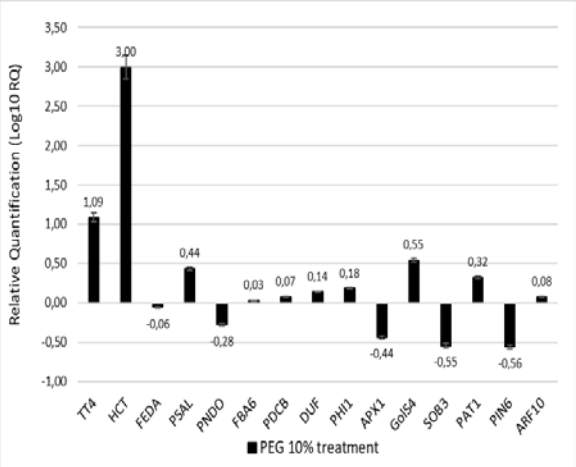


Figure 6. The expression analysis results of fifteen selected DEGs by qRT-PCR and using the *MaACT* and *MaBT* as housekeeping genes.

4. Discussion

Water stress has become a critical factor for the growth of banana plants, and caused substantial changes in their growth, morphological features, and biochemistry reactions (Surendar *et al.,* 2013). Chlorosis in shoots or leaves are indicated with the loss of their green color and turned into pale (Ahmed and El-Sayed, 2021). In banana plantlets the chlorosis in leaves seemed to have occurred as a result of the deterioration of chlorophyll and other photosynthetic pigments (Vergeiner *et al*., 2013). It was reported that lack of sufficient water supply decreased the total chlorophyll content and declined yield of banana production in the field (Ramos *et al*., 2019, and Uwimana *et al*, 2021). The transcriptomic analysis would be able to discover the reason (Bashir *et al.*, 2021).

The results of transcriptomic profiling showed that 78% (101,962 genes) of the assembled contigs were mapped to the gene model of the banana genome reference available in CIRAD library. It was indicated that the transcriptome profile was quite unique between different cultivars of banana. There are about 22% (45,849 genes) of the *M. acuminata* cv Barangan Merah that did not match any gene in the previously published *M. acuminata* cv 'DH Pahang' at CIRAD. The difference, however, could also be contributed by the different sample types where the RNA was resourced (Wang *et al*., 2020). The quite large functional variety of the top 100 upregulated genes indicated that once challenged with water deficit conditions, the banana plantlets simultaneously activated many 'strategies' to maintain all the cellular basic functions by protecting cellular components from damage while minimizing further water loss and detoxifying the cells from the free radicals (Muthusamy *et al.*, 2016).

The 10% PEG treatment evidently enhanced expression of genes related with biosynthesis of amino acids including proline, glutamine, glutathione, and anthocyanin. The result indicated that one of the primary responses of the drought-stressed Barangan Merah banana cells was to actively produce those amino acids to maintain cellular osmotic balance as well as to chelate the reactive oxygen species (ROS) and other free radicals (Mahdid *et al.*, 2020). The increase of amino acid production and protein formation is known to be related with the biosynthesis of signaling proteins, which play significant roles in response to abiotic stresses, including drought (Bashir *et al*., 2021). Furthermore, signaling proteins are involved in various biological functions of growth and development of plants cells of bananas (Mattos-Moreira *et al*., 2018). Similar results were also reported (Xu *et al*., 2019). The PEG-induced water stress also affected the contents of soluble proteins and gave an impact on increasing the biosynthesis

of proline, and free amino acid in banana plantlets (Amnan *et al*., 2021).

Our data showed a drastic turndown in the expression of genes responsible for the biosynthesis of both the photosynthesis apparatus and the enzymes that catalyzed the process. When water, as one of the primary components in photosynthesis, was diminished, the plant adjusted by down-regulating the relevant genes for photosynthesis, preserving energy for other primary functions. Similarly, previous studies reported a large turndown of photosynthetic genes in tangor (Xiao *et al*., 2017), bermudagrass (Yuan *et al*, 2021), and several okra varieties (Ahmed and El-Sayed., 2021) when the plants are under drought stress. It was also reported in rice plants that a major reduction in photosynthesis rates was related to the decrease in photosynthesis enzyme activities and oxidative damage to the chloroplast (Wada *et al.* 2019). A reduction in photosynthesis rate as a response to drought stress was reportedly occurred through the stomatal- and non-stomatal-routes in potato (Chen *et al*., 2020). In the lower level of drought, the stomatal conductance was lowered down upon the sensing of water deficit, resulting in stomatal closure, and causing the decrease of photosynthesis rate (Yuan *et al*., 2021). In a higher drought stress, the free radicals accumulate to a level that exceeds the capacity of cellular antioxidants to neutralize. This signals the cell to repress the production of a core protein of photosystem II, causing further reduction in photosynthesis (Wang *et al*., 2020).

In the present study, it was indicated that the plant perceives the 10% PEG treatment as severe water stress due to at least two reasons: first, the massive downregulation of chloroplast structural genes following drought exposure; second, the high expression of the stress-marker genes, such as *superoxide dismutase (SOD)* and *ascorbate peroxidase (APX)*, as well as the antioxidant genes. In addition to photosynthesis reduction, a large amount of stress responding genes was highly increased upon drought treatment. Interestingly, the genes that were activated upon drought stress were the genes that are also responsive to other kinds of abiotic stimuli, such as light, radiation, temperature (Yang *et al*., 2015), chemicals (Xu *et al*., 2019) and hypoxia (Ilyas *et al*., 2021). These stress-responsive gene families produce two types of products. The first types are proteins that are involved in the protection of the cells and in the regulation of signal transduction of stress-related-pathways, such as *heat shock proteins (HSP)*, *chaperons, late-embryogenesis abundant (LEA)* proteins (Oguz *et al.*, 2022). Second products are proteins that involve in further regulation of signal transduction and stress-responsive gene expression (Kimotho *et al*., 2019), such as *Transcription Factors (TFs)* and *dehydration responsive elements (DRE)*. Our DEG results also identified 1 *heat shock proteins (HSP)* genes, 5 chaperon genes, 4 reactive oxygen species (ROS)-responsive genes, and 47 transcription factors. In fact, water shortage signals the cells, through activation of abscisic acid (ABA)-dependent and ABA-independent regulatory systems, to activate the expressions of various drought related genes. Similar results were reported in various plants when the plants were exposed to a water stress condition (Seleiman *et al.*, 2021).

The reduction in photosynthesis rate consequently reduced the production of cellular energy resources. In addition, water-deficit stress causes large energy consumption. Many studies showed that plants compensate for this energy-demanding condition by increasing their cellular respiration (Yuan *et al.,* 2021). It demonstrated upregulation of genes encodes for mitochondrial components as well as the genes playing a role in glycolysis, electron transport chain, and electron carrier activity. Eight genes related to 'mitochondrial structures', four genes were assigned to 'mitochondrial respiratory chain complex', three genes of 'cytochrome c, and nine genes related to 'glycolytic activity' were identified in this study, which demonstrated a substantial raise in oxidative respiration process following water-deficit stress. In addition to oxidative respiration, plant under drought stress also reportedly exhibited alternative respiration through fermentation in order to survive the harsh condition (Hu *et al*., 2017). In line with this, four pyruvate kinase genes and eight alcohol dehydrogenase genes were identified, both are important enzymes in the anaerobic respiration pathway. This may imply that the stressed-banana employs the anaerobic respiration, in addition to the aerobic respiration, to cope with the water scarcity (Mahdid *et al*., 2020). Our data showed that there are so many changes in other processes in banana plant that consistently occurred in all PEG treatment groups, such as polysaccharide metabolic and catabolic processes, multicellular organismal development, intracellular and transmembrane transports, post-translational protein modification, cell surface receptor-linked signaling pathway, and regulation of cellular chemical homeostasis (Ahmed and El-Sayed, 2021).

Plants are known to produce a wide array of secondary metabolites as a defense mechanism against environmental stresses. Drought is also able to influence the biosynthesis of secondary metabolites, that are positively correlated with the increments of antioxidants (Al-Gabbiesh *et al.*, 2015). Phenylpropanoid biosynthesis pathway, in particular, has been reported to be highly activated, following harsh abiotic stresses including heavy metal, salinity, heat, cold, UV radiations, and drought, leading to the accumulation of polyphenolic compounds including flavonoids (Šamec *et al*., 2021). Anthocyanin, carotenoid, flavonoid, and phenolic compounds are known as the antioxidants, which are important in protecting water-stressed cells from the damage caused by the ROS (Ahad *et al.,* 2018). Among others, flavonoids have been suggested to contribute in protecting plants from abiotic disturbances, such as drought, root zone salinity, UV-radiation, and scavenging the harmful ROS (Xu *et al*., 2020). Biochemical and molecular mechanisms of flavonoid and phenyl-propanoid accumulation in response to abiotic stress have been well reviewed in various plants (Bashir *et al.*, 2021).

The DEG result identified 47 transcription factors which were affected by the 10% PEG-induced water stress. The central role of transcription factors (TF) in regulating the transcription of stress-responsive genes following environmental stresses has been widely reported and reviewed (Joshi *et al*., 2016). The identification of those TF families in our studies indicated that, like many other plants, *Musa acuminata* also uses both ABA-dependent and ABA-dependent routes to activate the expression of stress-responsive genes (Hrmova and Hussain 2021). Involvement of TF families, such as

WRKY, *MYB*, *bZIP*, *ABF*, *ABRE*, and *DRE*, in response to abiotic stresses had been reported in bananas. García-Laynes *et al.* (2022) identified the *MaWRKY* family genes from the wild banana *Musa acuminata* ssp. *malaccensis*, which is known as a progenitor of most banana cultivars, and resistant to several diseases and environmental disturbances. Their study discovered that those *MaWRKY* genes showed distinctive expressions in banana plants in response to environmental stresses. The *WRKY* transcription factors (TFs) has been known to encode functional transcription factors (Chen *et al.*, 2015), and play a part in plant defense responses through phytohormone the signaling pathways (Yang *et al*., 2015). Moreover, there had been many identified TF families from *Musa acuminata* that were highly affected by the drought conditions. Interestingly, hormone-activated TFs, such as ethylene response factor (ERF, 4 genes) and auxin-induced TFs (2 genes), were among the TF families induced by water-deficit. The result implied that banana plantlets also used auxin and ethylene, in addition to abscisic acid, to signal the activation of stress responses genes (Hu *et al.*, 2015). Our GO analysis results also showed enriched numbers of genes that take parts in post-embryonic- and plant organ development. Another finding that we found intriguing was the identification of a gene encodes for WUSCHEL-related homeobox (WOX), a TF family that is known to play important roles in determining cell fates during embryogenesis, and all other stages of plant development even under water stress. In this study, it was discovered that, like other plants, banana plantlets responded to water stress through a complex metabolic and signaling networks (Oguz *et al.*, 2022).

As shown in Figure 6, in general, the confirmation of gene expressions of 15 selected annotated DEGs showed similar trends of expressions between qRT-PCR data and RNA-seq data. These 15 DEGs were selected for their potentially key roles in some important bioprocesses, which were affected by water stress. Those genes are identified and known to be involved in photosynthesis (*MaFEDA, MaPSAL,* and *MaPNDO*), biosynthesis of flavonoid and antioxidants (*MaTT4* and *MaHCT*), glycolysis (*MaFBA6* and *MaPDCB*), respond to osmotic stress (*MaDUF*, *MaPHI1*, *MaAPX1,* and *MaGolS4*), organ development (*MaSOB3, MaPAT1*and *MaPIN6*), and as a transcription factor (*MaARF10*). Gene functions of the 15 selected DEG are listed in Supplementary Data Table S.1. In several points, there were some dissimilarities between expression results based on the transcriptome analysis and the qRT-PCR assay, for instance the *MaGolS4, MaSOB3*, and *MaPAT1* genes. A similar research result was reported in banana transcriptomic analysis by Hu *et al.* (2017). They suggested that there were some possible reasons for discrepancies. The first possibility might be due to the fact that genes have different alternative forms, where the RNA-seq might be able to capture the expression of all alternative forms for a gene, while the qRT-PCR assay might capture the expression of only one alternative form. Secondly, the RNA-seq and qRT-PCR seemed to exhibit consistent results for genes with high significance expressions and might not be persistent for genes with low expressions (Hu *et al.*, 2017). Taken together, because of the important functions of these genes in banana defense mechanism, further studies are still needed.

5. Conclusion

Morphological changes occurred in plantlets after four weeks of PEG exposure. The addition of PEG caused a lack of sufficient water and seemed to be corresponding to the changes in transcriptome profiles. A transcriptome library of 129,701 annotated genes, and the expression profile, of banana (*Musa acuminata* cv 'Barangan Merah') has been established. Transcriptomic analysis revealed eight major biological processes were highly affected by the drought stress in banana plantlets, those are: 1) photosynthesis, 2) cellular redox balance; 3) cellular components stability; 4) cellular energy preservation; 5) metal ion homeostasis; 6) hormonal-activated signaling pathways; 7) production of transcription factors; and 8) organ development. This study found that exposure to water stress highly influenced primary and secondary metabolism in banana plantlets. In a relatively smaller extent, other processes that seemed to be affected are cell wall re-organization, plant hormone production, transmembrane signaling pathways and secondary metabolite biosynthesis. The high induced phenylpropanoid biosynthesis and flavonoid pathways suggest that banana produce flavonoid compounds in response to water stress, which evident and identities need to be further investigated. A total of 47 genes encode for transcription factor (TF) were identified. They belong to at least 26 TF families, including five families that are typical for stress-responsive genes families (MYB, WRKY, bZIP, ABF, DRE), auxin- and ethylene-activated TFs and WUSCHEL-related homeobox (WOX). Further transcriptomic analyses are still needed to reveal the effect of water stress on major bioprocesses in detail.

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Conflict of interest

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

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SUPPLEMENTARY TABLES

Table S.1. List of 17 selected gene primers used for validation using qRT-PCR assay including housekeeping genes.

Gene Symbol	Function	Primer Sequence (Forward)	Primer Sequence (Reverse)	Product Size (bp)
MaACT	Actin histone-lysine N-methyltransferase setd3	CTGACTGGCAGCAGGACATA	CCAAATCGTGCCTTTGAACT	162
MaBT	Beta tubulin (housekeeping)	AGTCCGGAGCTTCAACCTTT	ACGCTGACGATGGAGAAGAC	221
MaTT4	Chalcone and stilbene synthase	CTCCCAACCTCTACGAGCAG	GGGTCCATGTAGGAGCACAT	267
MaHCT	Hydroxycinnamoyl-CoA shikimate transferase	ATGGTGGAAGTGGTGGAATC	TTGAGCAGCTGTACGGAGAA	167
MaFEDA	2-Fe-2S ferredoxin	TTGCCATCTCTCCCTGTCTT	GGCATTCGATCACCTTCTCT	214
MaPSAL	Photosystem I subunit l	GCATCTCACGAACACCATTG	GATGGGCTGAATCACTTGGT	196
MaPNDO	Pyridine nucleotide-disulfide oxidoreductase	GCTTTCTCCAGCATCAAAGG	CCCATTCCTCCTTCGACATA	216
MaFBA6	Aldolase superfamily protein 6	CTCAGGAGGGCAGAGTGAAG	CTCGCCTTCTCGACATTCTC	162
MaPDCB	Thiamine pyrophosphate dependent pyruvate decarboxylase family protein B	TGTGCTTCATCGAGGTCATC	AGTCTCGGACGCAAGAACAT	215
MaDUF	2-Aminoethanethiol dioxygenase, putative	TCCAGGCATGACGGTATTCAC	TGAGGCACATTGACCCAGTC	78
MaPHI1	Phosphate-responsive 1 family protein	TAACACGAACCCAAGAAGCG	CCCACCACCATATTCTGCTAAG	95
MaAPX1	Ascorbate peroxidase 1	ACGATGTGGTGTCAAAGACG	GTATGTCAAGATGGGGAACTGC	140
MaGolS4	Galactinol synthase 4	TCGAAGAAGGTAAGCAGGTCTC	CACTGGAAAGGAAGCTAACATGG	140
MaSOB3	Putative AT-hook DNA-binding family protein	TCGCAGCCACATTCTTGAAC	GCAATCCTTCTGTGACCAATCG	96
MaPAT1	GRAS family transcription factor	ATCGTCAATCCCTGTGATCCG	TGACTTCCAGATTGCTCAAGGG	103
MaPIN6	Auxin efflux carrier family protein	GGATCTCACAGTTTCCTTCGTTG	ACATCACGGTGTAGAAGTCCTC	71
MaARF10	Auxin response factor 10	AATGTGAACCGTGTGAACCC	AGAAGGGAGCAAGATGGATAGC	73

Table S.2. List of the 94 annotated genes, out of 233 genes, that were consistently downregulated in all levels of PEG treatments (PEG 2.5, PEG7, and PEG10).

Contig ID *M.acuminata* 'Barangan'	GO annotation		
	Biological process	Cellular components	Molecular function
MaB_DN55073_c0_g1	regulation of endosperm development; regulation of starch biosynthetic process	chloroplast	amylopectin binding; maltoheptaose binding; starch binding
MaB_DN54882_c0_g1	actin filament-based movement; cell division; fruit development; Golgi localization; gynoecium development; mitochondrion localization; post-embryonic development; root hair elongation; trichome morphogenesis	myosin complex; plasmodesma; root hair tip; transport vesicle	actin binding; ATP binding; calmodulin binding; motor activity
MaB_DN84725_c0_g1	leaf-, floral-, fruit- morphogenesis; microtubule cytoskeleton organization; oxidation-reduction process; regulation of cell shape; regulation of epidermal cell division and differentiation; regulation of trichome morphogenesis; response to osmotic stress; response to salt stress	cytoplasmic stress granule; cytosol; microtubule; trans-Golgi network	identical protein binding; NAD binding; protein homodimerization activity
MaB_DN71017_c0_g1	cation transport; cellular metal ion homeostasis; cellular response to phosphate starvation; meristem maintenance; pollen germination; pollen maturation; stem cell fate determination	endoplasmic reticulum membrane; integral component of membrane; plasma membrane	ATP binding; ATPase activity; metal ion binding
MaB_DN8953_c0_g2	defense response to bacterium; negative regulation of translation; nuclear-transcribed mRNA poly(A) tail shortening	CCR4-NOT core complex; nucleus; P-body	3'-5' exonuclease activity; metal ion binding; poly(A)-specific ribonuclease activity; RNA binding
MaB_DN36213_c0_g1	lysyl-tRNA aminoacylation	cytosol	ATP binding; lysine-tRNA ligase activity; tRNA binding
MaB_DN27607_c0_g4	carbon utilization	chloroplast stroma	carbonate dehydratase activity; zinc ion binding
MaB_DN6111_c0_g2	photosynthesis	chloroplast thylakoid membrane; integral component of membrane; photosystem I reaction center	.
MaB_DN31557_c0_g1	signal transduction	.	ATP binding; protein serine/threonine kinase activity
MaB_DN75200_c0_g1	endocytosis; lipid transport; viral process	endoplasmic reticulum; endosome membrane; integral component of membrane; plasma membrane	lipid binding; metal ion binding
MaB_DN3084_c0_g1	protein ubiquitination; response to cadmium ion; response to chitin	.	metal ion binding; ubiquitin-protein transferase activity
MaB_DN5666_c0_g1	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus	chloroplast envelope; chloroplast thylakoid membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding
MaB_DN62333_c0_g1	phosphatidylinositol phosphorylation; phosphatidylinositol-mediated signaling	chloroplast; cytoplasm; membrane; plasma membrane	1-phosphatidylinositol 4-kinase activity
MaB_DN344_c0_g4	photosynthesis, light harvesting; protein-chromophore linkage	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding

MaB_DN7600_c0_g1	amino acid transmembrane transport; amino acid transport	integral component of membrane; plasma membrane	amino acid transmembrane transporter activity; primary active transmembrane transporter activity; symporter activity
MaB_DN345_c0_g2	.	.	ATP binding; ubiquitin conjugating enzyme activity
MaB_DN16594_c1_g1	cellular copper ion homeostasis; response to light intensity; zinc ion homeostasis	chloroplast; chloroplast envelope; chloroplast inner membrane	ATP binding; ATPase activity; cadmium transmembrane transporter activity, phosphorylative mechanism; metal ion binding; ATPase-coupled zinc transmembrane transporter activity
MaB_DN17080_c0_g2		nucleus	DNA binding; metal ion binding
MaB_DN6425_c0_g1	transport of virus in host, cell to cell; viral entry into host cell; viral penetration into host nucleus	host cell	aspartic-type endopeptidase activity; nucleic acid binding; RNA-directed DNA polymerase activity; RNA-DNA hybrid ribonuclease activity; zinc ion binding
MaB_DN82953_c0_g1	histone H3-K4 methylation	histone methyltransferase complex	histone binding
MaB_DN26630_c0_g1	intracellular signal transduction; protein phosphorylation	cytoplasm; nucleus	ATP binding; protein serine/threonine kinase activity
MaB_DN904_c0_g1	.	chloroplast stroma	ATP binding
MaB_DN68858_c0_g1	iron ion homeostasis	integral component of membrane	cadmium ion transmembrane transporter activity; manganese ion transmembrane transporter activity
MaB_DN95436_c0_g1	long-day photoperiodism; flowering; regulation of timing of transition from vegetative to reproductive phase	cytoplasm; nucleus; perinuclear region of cytoplasm; plasma membrane	transcription factor binding
MaB_DN72306_c0_g1	unsaturated fatty acid biosynthetic process	integral component of membrane	oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water
MaB_DN81905_c0_g1	.	integral component of membrane; plasma membrane	ATP binding; protein serine/threonine kinase activity
MaB_DN78426_c0_g1	peptidyl-L-cysteine S-palmitoylation; protein targeting to membrane	endoplasmic reticulum; Golgi apparatus; integral component of membrane; plasma membrane	protein-cysteine S-palmitoyltransferase activity
MaB_DN7935_c0_g1	photosynthesis, light harvesting; protein-chromophore linkage	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding
MaB_DN58280_c0_g1	.	integral component of membrane; plasma membrane	ATP binding; protein serine/threonine kinase activity
MaB_DN904_c0_g2	.	chloroplast stroma	ATP binding
MaB_DN8360_c0_g1	.	.	ATP binding; helicase activity; RNA binding
MaB_DN14270_c0_g1	polar nucleus fusion; rRNA pseudouridine synthesis; snRNA pseudouridine synthesis	box H/ACA snoRNP complex; box H/ACA telomerase RNP complex; Cajal body; nucleolus	box H/ACA snoRNA binding; telomerase RNA binding
MaB_DN10259_c0_g2	.	nucleus	AT DNA binding; DNA-binding transcription factor activity
MaB_DN78376_c0_g1	defense response to Gram-negative bacterium; floral organ abscission; lateral root morphogenesis; leaf abscission; pectin catabolic process; protein autophosphorylation; regulation of gene expression	integral component of membrane; plasma membrane	ATP binding; protein serine/threonine kinase activity; transmembrane receptor protein tyrosine kinase activity

MaB_DN65589_c0_g1	defense response to bacterium, fungus; regulation of defense response; response to salicylic acid	nucleus	DNA-binding transcription factor activity; sequence-specific DNA binding
MaB_DN7935_c1_g1	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding
MaB_DN43104_c0_g1	amino acid transmembrane transport; gamma-aminobutyric acid transport	integral component of membrane; plasma membrane	amino acid transmembrane transporter activity; gamma-aminobutyric acid transmembrane transporter activity
MaB_DN67987_c0_g1	trichome branching	.	calcium ion binding
MaB_DN12891_c0_g1	.	chloroplast stroma	ATP binding
MaB_DN7159_c0_g1	response to chitin	nucleus	calmodulin binding; DNA-binding transcription factor activity; transcription regulatory region DNA binding
MaB_DN78578_c0_g1	peptidyl-pyroglutamic acid biosynthetic process, using glutaminyl-peptide cyclotransferase	endoplasmic reticulum membrane; integral component of membrane; plasma membrane	glutaminyl-peptide cyclotransferase activity
MaB_DN1568_c0_g2	chlorophyll biosynthetic process; photosynthesis	chloroplast	protochlorophyllide reductase activity
MaB_DN58553_c0_g1	.	.	transferase activity, transferring hexosyl groups
MaB_DN7935_c2_g1	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem; photosystem II; plastoglobule	chlorophyll binding; metal ion binding
MaB_DN89859_c0_g1	cell cycle; multicellular organism development; response to abscisic acid	cytoplasm	.
MaB_DN99671_c0_g1	cellular response to phosphate starvation; phosphate ion transport	Golgi apparatus; integral component of membrane; plasma membrane; trans-Golgi network	inositol hexakisphosphate binding; phosphate ion transmembrane transporter activity
MaB_DN62890_c0_g1	cytokinin-activated signaling pathway; phosphorelay signal transduction system; phosphorylation	cytoplasm; nucleus	histidine phosphotransfer kinase activity; protein histidine kinase binding
MaB_DN47361_c0_g1	photorespiration	integral component of membrane; mitochondrial membrane; mitochondrial respiratory chain complex I; respiratory chain complex I	NADH dehydrogenase (ubiquinone) activity
MaB_DN12562_c0_g2	.	.	serine-type carboxypeptidase activity
MaB_DN36962_c0_g1	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding
MaB_DN66931_c0_g1	.	integral component of membrane; plasma membrane	transmembrane transporter activity
MaB_DN75006_c0_g1	clathrin coat assembly; clathrin-dependent endocytosis; pollen tube growth; protein localization to plasma membrane; vesicle budding from membrane	clathrin-coated pit; clathrin-coated vesicle; Golgi apparatus; plasma membrane; pollen tube	1-phosphatidylinositol binding; clathrin heavy chain binding; phosphatidylinositol-4,5-bisphosphate binding; SNARE binding
MaB_DN1777_c1_g1	translation	cytosolic large ribosomal subunit; plasma membrane	mRNA binding; structural constituent of ribosome

MaB_DN63_c1_g1	.	.	calcium ion binding
MaB_DN10464_c0_g3	autophagosome assembly; protein autophosphorylation; protein transport; regulation of autophagy	autophagosome; cytoplasmic vesicle; cytosol; membrane; phagophore assembly site	ATP binding; protein serine/threonine kinase activity
MaB_DN12135_c0_g1	defense response signaling pathway; detection of bacterium; immune response-regulating signaling pathway; plant-type hypersensitive response; regulation of anion channel activity	endomembrane system; integral component of membrane; plasma membrane	ATP binding; protein serine/threonine kinase activity; transmembrane receptor protein kinase activity
MaB_DN39798_c0_g1	transmembrane transport	integral component of membrane; plasma membrane	ATP binding; ATPase-coupled transmembrane transporter activity
MaB_DN7546_c0_g2	pollen tube growth	.	carboxylic ester hydrolase activity; methyl indole-3-acetate esterase activity
MaB_DN11415_c0_g1	abscisic acid-activated signaling pathway; glucosinolate metabolic process	nucleus	DNA-binding transcription factor activity; sequence-specific DNA binding
MaB_DN6765_c0_g3	photosynthesis; light harvesting in photosystem I, photosystem II; protein-chromophore linkage; regulation of stomatal movement; response to abscisic acid; response to high light stimulus	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule; thylakoid membrane	chlorophyll binding; metal ion binding; protein domain specific binding
MaB_DN344_c0_g3	photosynthesis, light harvesting in photosystem; protein-chromophore linkage; response to light stimulus	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding
MaB_DN27607_c0_g1	carbon utilization	chloroplast stroma	carbonate dehydratase activity; zinc ion binding
MaB_DN8953_c0_g1	defense response to bacterium; negative regulation of translation; nuclear-transcribed mRNA poly(A) tail shortening	CCR4-NOT core complex; nucleus; P-body	3'-5' exonuclease activity; metal ion binding; poly(A)-specific ribonuclease activity; RNA binding
MaB_DN36120_c0_g1	pentose-phosphate shunt; reductive pentose-phosphate cycle	chloroplast thylakoid membrane; cytosol	calcium ion binding; cobalt ion binding; manganese ion binding; transketolase activity
MaB_DN59498_c0_g1	.	anchored component of membrane; anchored component of plasma membrane	electron transfer activity; metal ion binding
MaB_DN13736_c0_g1	.	integral component of membrane	carbon-sulfur lyase activity; transaminase activity
MaB_DN2207_c0_g3	photosynthetic electron transport in photosystem I; photosynthetic NADP+ reduction; photosystem I stabilization	chloroplast envelope; chloroplast membrane; photosystem I; chloroplast thylakoid membrane; integral component of membrane	.
MaB_DN76923_c0_g1	abscisic acid-activated signaling pathway; response to abscisic acid; response to salt stress; response to water deprivation	nucleus	DNA-binding transcription factor activity; transcription regulatory region sequence-specific DNA binding
MaB_DN45814_c0_g1	.	cell wall; extracellular region	.
MaB_DN59396_c0_g1	.	extracellular space	copper ion binding; oxidoreductase activity
MaB_DN13250_c0_g1	.	chloroplast thylakoid membrane; integral component of membrane	.
MaB_DN2764_c1_g8	auxin-activated signaling pathway, negative regulation of transcription	nucleus	DNA-binding transcription factor activity; sequence-specific DNA binding

MaB_DN80742_c0_g1	.	membrane	heme binding; iron ion binding; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen
MaB_DN64755_c0_g1	hydrogen peroxide catabolic process; response to oxidative stress	extracellular region	heme binding; metal ion binding; peroxidase activity
MaB_DN34665_c0_g1	photosynthesis; pyruvate metabolic process	chloroplast	ATP binding; kinase activity; metal ion binding; pyruvate, phosphate dikinase activity
MaB_DN67584_c0_g1	amyloplast organization; cell division; embryo development ending in seed dormancy; embryonic axis specification; late endosome to vacuole transport, receptor-mediated endocytosis	endoplasmic reticulum; endosome membrane; Golgi apparatus; intracellular membrane-bounded organelle; late endosome membrane; trans-Golgi network; vacuolar membrane	.
MaB_DN7510_c0_g1	.	cytoplasm	5'-nucleotidase activity; metal ion binding; nucleotide binding
MaB_DN82130_c0_g1	defense response; response to biotic stimulus	extracellular region; vacuole	.
MaB_DN7968_c0_g2	ribosomal large subunit assembly	preribosome, large subunit precursor	.
MaB_DN22837_c0_g1	cell wall biogenesis; cell wall organization; xyloglucan metabolic process	apoplast, cell wall	hydrolase activity, hydrolyzing O-glycosyl compounds; xyloglucan: xyloglucosyl transferase activity
MaB_DN16139_c0_g1	regulation of transcription	.	DNA-binding transcription factor activity; metal ion binding
MaB_DN31_c2_g1	defense response to fungus; induced systemic resistance; response to abscisic acid; response to cold; response to salt stress	chloroplast outer membrane; endoplasmic reticulum; extracellular region; plant-type cell wall; plasmodesma	.
MaB_DN77256_c0_g1	defense response to fungus; response to abscisic acid; response to glucose; response to salt stress	mitochondrion	.
MaB_DN64112_c0_g1	cell division; phloem or xylem histogenesis; procambium histogenesis	nucleus	DNA binding
MaB_DN7987_c0_g1	phenylpropanoid metabolic process	.	4-coumarate-CoA ligase activity; ATP binding
MaB_DN45737_c0_g1	RNA modification	.	zinc ion binding
MaB_DN36169_c0_g1	.	.	electron transfer activity; metal ion binding
MaB_DN90833_c0_g1	copper ion transmembrane transport; photosynthetic electron transport chain	chloroplast envelope; chloroplast membrane; chloroplast stroma; integral component of membrane; plastid	ATP binding; copper chaperone activity; copper transmembrane transporter activity, phosphorylative mechanism
MaB_DN36962_c0_g2	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage	chloroplast thylakoid membrane; integral component of membrane; photosystem I-II; plastoglobule	chlorophyll binding; metal ion binding
MaB_DN7935_c3_g1	photosynthesis; light harvesting; protein-chromophore linkage	chloroplast thylakoid membrane; integral component of membrane; photosystem I-II	chlorophyll binding; metal ion binding
MaB_DN7935_c4_g1	photosynthesis; light harvesting; protein-chromophore linkage	chloroplast thylakoid membrane; integral component of membrane; photosystem I-II	chlorophyll binding; metal ion binding
MaB_DN22217_c1_g2	photosynthesis	chloroplast photosystem I	protein domain specific binding
MaB_DN17076_c0_g1	defense response; ethylene-activated signaling pathway	nucleus	DNA binding; DNA-binding transcription factor activity
MaB_DN95147_c0_g1	poly(A)+ mRNA export from nucleus	cell	DNA binding; metal ion and mRNA

				binding

Table S.3. List of the 100 genes with the highest upregulation following the 10% PEG treatment.

Contig ID *M.acuminata* 'Barangan'	ID M acuminata 'Pahang'	GO annotation		
		Cellular component	Molecular function	Biological process
MaB_DN477_c0_g1	Ma06_p06180.1	integral component of membrane	.	nodulation
MaB_DN553_c0_g1	Ma06_p06180.1	integral component of membrane	.	nodulation
MaB_DN2532_c0_g2	Ma05_p07040.1	cytoplasm	L-ascorbate peroxidase activity	hydrogen peroxide catabolic process; response to oxidative stress
MaB_DN1283_c1_g1	Ma09_p30640.1	.	.	.
MaB_DN32_c0_g2	Ma08_p33280.1	.	chitinase activity	chitin catabolic process; polysaccharide catabolic process
MaB_DN37218_c0_g1	Ma09_p11800.1	.	.	.
MaB_DN2428_c0_g2	NA	.	.	.
MaB_DN955_c0_g1	Ma08_p23240.1	.	.	.
MaB_DN3418_c0_g1	Ma06_p06040.1	endoplasmic reticulum membrane	Iron ion binding; L-ascorbic acid binding; procollagen-proline 4-dioxygenase activity	peptidyl-proline hydroxylation to 4-hydroxy-L-proline
MaB_DN9130_c0_g1	Ma09_p30690.1	.	.	.
MaB_DN39392_c0_g1	Ma02_p04310.2	cytoplasm	metal ion binding; superoxide dismutase activity	.
MaB_DN176_c1_g1	Ma08_p30760.1	plasma membrane; vacuolar membrane	proton-transporting ATPase activity	.
MaB_DN1937_c0_g2	Ma04_p29690.1	.	.	.
MaB_DN4829_c0_g1	Ma05_p31910.1	.	.	.
MaB_DN20701_c0_g1	Ma01_p10230.1	endoplasmic reticulum lumen	protein disulfide isomerase activity	cell redox homeostasis
MaB_DN1912_c0_g2	Ma02_p03250.1	nucleolus; ribonucleoprotein complex	nucleosome binding; RNA binding	rRNA processing
MaB_DN138_c0_g2	Ma06_p03940.1	cell wall; extracellular region	.	defense response
MaB_DN402_c0_g1	Ma08_p20020.1	cytoplasm; microtubule; plasma membrane	GTP binding; structural constituent of cytoskeleton	microtubule cytoskeleton organization; mitotic cell cycle
MaB_DN8443_c0_g1	Ma03_p12340.1	cytoplasm; nucleus	kinase binding; metal ion binding; phosphatase binding	response to glucose; response to mannose; response to sucrose
MaB_DN2818_c0_g4	Ma11_p14250.1	endoplasmic reticulum	monooxygenase activity; oxidoreductase activity	anthocyanin-containing compound biosynthetic process
MaB_DN180_c0_g3	Ma05_p25540.1	U12-type spliceosomal complex; U5 snRNP	mRNA binding	spliceosomal snRNP assembly
MaB_DN12172_c0_g1	Ma07_p03130.1	chloroplast stroma; membrane	acyl-[acyl-carrier-protein] desaturase activity; metal ion binding; stearoyl-[acp] desaturase activity	fatty acid biosynthetic process
MaB_DN10862_c0_g1	Ma09_p24220.1	cytoplasm	pyruvate kinase activity	cellular response to insulin stimulus; glycolytic process
MaB_DN43140_c0_g1	Ma05_p26790.2	.	.	.
MaB_DN89_c0_g3	Ma08_p29910.1	cytoplasm	alcohol dehydrogenase (NAD) activity; zinc ion binding	.
MaB_DN277_c0_g2	Ma06_p11050.1	chloroplast stroma; chloroplast thylakoid	fructose-bisphosphate aldolase activity	fructose 1,6-bisphosphate metabolic process; gluconeogenesis; glycolytic process

MaB_DN7961_c0_g2	Ma09_p27320.1	.	.	.
MaB_DN21625_c0_g1	Ma08_p27150.1	.	.	.
MaB_DN9175_c0_g2	Ma07_p03130.1	chloroplast stroma; membrane	acyl-[acyl-carrier-protein] desaturase activity; metal ion binding; stearoyl-[acp] desaturase activity	fatty acid biosynthetic process
MaB_DN7723_c0_g1	Ma03_p05380.1	.	heme binding; iron ion binding; monooxygenase activity; oxidoreductase activity	.
MaB_DN767_c1_g2	Ma04_p40090.1	nucleosome; nucleus	DNA binding; protein heterodimerization activity	.
MaB_DN3032_c1_g1	Ma10_p15940.1	cell wall; extracellular region; vacuole	heme binding; metal ion binding; peroxidase activity	hydrogen peroxide catabolic process; response to oxidative stress
MaB_DN17547_c0_g1	Ma02_p09950.1	cytoplasm	alcohol dehydrogenase (NAD) activity; metal ion binding	.
MaB_DN999_c1_g1	Ma06_p07440.1	eukaryotic translation initiation factor 2B complex	mRNA binding; ribosome binding; translation initiation factor activity	.
MaB_DN1937_c0_g1	Ma04_p29690.1	.	.	.
MaB_DN2366_c0_g2	Ma08_p24610.1	cytosolic large ribosomal subunit	protein-containing complex binding; structural constituent of ribosome	response to anoxia; translational elongation
MaB_DN4517_c0_g1	Ma05_p31910.1	.	.	.
MaB_DN4934_c0_g2	Ma02_p00070.1	nucleus	DNA-binding transcription factor activity	ethylene-activated signaling pathway; regulation of root development; response to anoxia
MaB_DN4894_c0_g1	NA	.	.	.
MaB_DN3972_c0_g2	Ma06_p13180.1	cytoplasm; nucleus	metal ion binding	response to glucose; response to mannose; response to sucrose
MaB_DN75017_c0_g1	Ma01_p13850.2	box H/ACA snoRNP complex; box H/ACA telomerase RNP complex	box H/ACA snoRNA binding; telomerase RNA binding	polar nucleus fusion; rRNA pseudouridine synthesis; snRNA pseudouridine synthesis
MaB_DN9175_c0_g1	Ma07_p03130.1	chloroplast stroma; membrane	acyl-[acyl-carrier-protein] desaturase activity; metal ion binding; stearoyl-[acp] desaturase activity	fatty acid biosynthetic process
MaB_DN11566_c0_g2	Ma10_p21060.1	cytosol; nucleus	cysteine dioxygenase activity; metal ion binding	detection of hypoxia; peptidyl-cysteine oxidation; response to hypoxia
MaB_DN194_c0_g2	NA	.	.	.
MaB_DN8753_c0_g1	NA	.	.	.
MaB_DN27124_c0_g1	NA	.	.	.
MaB_DN436_c0_g3	Ma05_p28820.1	plasma membrane	FMN binding; NAD(P)H dehydrogenase (quinone) activity	cellular response to auxin stimulus; oxidation-reduction process
MaB_DN46343_c0_g1	NA	.	.	.
MaB_DN16664_c0_g1	NA	.	.	.
MaB_DN3310_c0_g1	Ma06_p21540.1	chloroplast	phosphopantetheine binding	fatty acid biosynthetic process
MaB_DN38_c0_g4	Ma05_p14530.1	cytoplasm; nucleus	protein tag	cellular protein modification process; mRNA splicing, via spliceosome
MaB_DN52337_c0_g1	NA	.	.	.
MaB_DN6388_c0_g1	Ma04_p23090.1	BRCA1-BARD1 complex; cytoplasmic ribonucleoprotein granule; ubiquitin ligase complex	kinase binding; metal ion binding; protein homodimerization activity; RNA binding; ubiquitin-protein transferase activity	cell cycle arrest; DNA repair; negative reg of mRNA 3'-end processing; negative reg of protein export from nucleus; positive reg of apoptosis; positive reg of

				protein catabolic process; protein ubiquitination
MaB_DN8337_c2_g1	Ma02_p07900.1	actin cortical patch; Arp2/3 protein complex	actin binding; ATP binding	complex-mediated actin nucleation; multicellular organism development
MaB_DN9186_c0_g1	Ma02_p22480.1	.	carbohydrate binding	.
MaB_DN5380_c2_g1	NA	.	.	.
MaB_DN95883_c0_g1	NA	.	.	.
MaB_DN3027_c0_g1	Ma10_p27760.1	transcription elongation factor complex	metal ion binding; RNA polymerase II complex binding	chromatin-mediated maintenance of transcription; transcription elongation from RNA polymerase II promoter
MaB_DN4811_c0_g2	Ma05_p20020.1	Golgi apparatus; integral component of plasma membrane; vacuolar membrane	myo-inositol:proton symporter activity	carbohydrate transport
MaB_DN10_c1_g1	Ma03_p24340.1	cytoplasm; nucleus	.	.
MaB_DN2929_c0_g2	Ma06_p29340.1	cytosol; eukaryotic translation initiation factor 3 complex	metallopeptidase activity; translation initiation factor activity	abscisic acid-activated signaling pathway; positive regulation of translational initiation; response to auxin; response to glucose, maltose, sucrose
MaB_DN39009_c0_g1	Ma02_p22100.1	cytoplasm; cytosol	ATP binding; kinase activity; magnesium ion binding; potassium ion binding; pyruvate kinase activity	cellular response to insulin stimulus; glycolytic process
MaB_DN15150_c0_g1	Ma08_p11550.1	.	.	.
MaB_DN3508_c0_g1	Ma09_p30630.1	.	.	.
MaB_DN3960_c1_g1	Ma01_p16820.1	cytoplasm; cytosol	glutathione transferase activity	glutathione metabolic process; toxin catabolic process
MaB_DN60512_c0_g1	NA	.	.	.
MaB_DN10999_c0_g2	NA	.	.	.
MaB_DN13031_c0_g1	NA	.	.	.
MaB_DN1352_c0_g1	NA	.	.	.
MaB_DN73222_c0_g1	Ma03_p19090.1	.	.	.
MaB_DN15289_c0_g1	Ma02_p18370.1	nucleus	DNA binding; metal ion binding	jasmonic acid mediated signaling pathway; leaf senescence
MaB_DN2680_c0_g2	Ma04_p37480.1	cell wall; cytosolic small ribosomal subunit	structural constituent of ribosome	translation
MaB_DN4901_c1_g2	Ma11_p05230.1	nucleus	DNA binding; DNA-binding transcription factor activity	ethylene-activated signaling pathway; regulation of root development; response to anoxia
MaB_DN6967_c0_g5	NA	.	.	.
MaB_DN13691_c0_g1	Ma02_p05260.1	chloroplast	6-phosphogluconolactonase activity	carbohydrate metabolic process; pentose-phosphate shunt
MaB_DN5380_c1_g1	NA	.	.	.
MaB_DN578_c0_g1	Ma01_p07620.1	.	.	.
MaB_DN123_c0_g1	Ma02_p03590.1	ribosome	rRNA binding; structural constituent of ribosome	translation
MaB_DN53893_c0_g1	Ma01_p05190.1	mitochondrion	metal ion binding; NADH dehydrogenase (ubiquinone) activity; quinone binding	.
MaB_DN55569_c0_g1	Ma02_p18200.1	nucleus	DNA-binding transcription factor activity; protein self-association	flower development; photomorphogenesis; vegetative to reproductive phase transition of meristem

MaB_DN25760_c0_g1	Ma09_p20790.1	.	.	.
MaB_DN8892_c0_g2	Ma09_p19760.1	nucleus	DNA-binding transcription factor activity	circadian rhythm; flower development; negative regulation of gene expression; phosphorelay signal
MaB_DN19846_c0_g1	NA	.	.	.
MaB_DN43068_c0_g1	NA	.	.	.
MaB_DN44820_c0_g1	Ma08_p29910.1	cytoplasm	alcohol dehydrogenase (NAD) activity; zinc ion binding	.
MaB_DN12210_c0_g1	NA	.	.	.
MaB_DN43870_c0_g1	Ma08_p06620.2	.	.	.
MaB_DN46215_c0_g1	Ma02_p02500.1	integral component of membrane	metal ion binding	oxidation-reduction process
MaB_DN92475_c0_g1	Ma04_p27850.1	.	.	.
MaB_DN3655_c0_g2	Ma02_p19680.1	nucleus	NAD+ ADP-ribosyltransferase activity	multicellular organism development
MaB_DN423_c0_g2	mipo3_t00160.1	mitochondrion	.	.
MaB_DN46664_c0_g1	Ma10_p03280.2	.	.	.
MaB_DN6373_c0_g1	Ma05_p27790.1	cytoplasm	fructose-bisphosphate aldolase activity	fructose 1,6-bisphosphate metabolic process; gluconeogenesis; glycolytic process
MaB_DN6458_c0_g1	Ma06_p12350.2	.	sucrose synthase activity	sucrose metabolic process
MaB_DN6560_c0_g1	Ma08_p17730.1	nucleus	sequence-specific DNA binding	negative regulation of transcription, DNA-templated
MaB_DN81145_c0_g1	Ma01_p11690.2	.	.	chromatin organization; regulation of gene expression; epigenetic
MaB_DN98221_c0_g1	NA	.	.	.
MaB_DN1361_c0_g2	Ma05_p09360.1	.	1-aminocyclopropane-1-carboxylate oxidase activity; dioxygenase activity; L-ascorbic acid binding; metal ion binding	cellular response to fatty acid; cellular response to iron ion; defense response; ethylene biosynthetic process
MaB_DN17680_c0_g1	Ma04_p17320.2	chloroplast; clathrin coat of trans-Golgi network vesicle; plasma membrane	clathrin light chain binding; structural molecule activity	intracellular protein transport; receptor-mediated endocytosis
MaB_DN25228_c0_g1	NA	.	.	.

Table S.4. List of the 100 genes with the most <u>downregulation</u> following the 10% PEG treatment.

Contig ID M.acuminata 'Barangan'	ID M acuminata 'Pahang'	GO annotation		
		Cellular component	Molecular function	Biological process
MaB_DN766_c0_g1	Ma05_p08930.1	chloroplast envelope; chloroplast thylakoid membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus
MaB_DN1015_c0_g2	Ma03_p12880.1	apoplast, cell wall; chloroplast outer membrane; endoplasmic reticulum; plasmodesma	protein self-association	cellular response to cold; cold acclimation; defense response to fungus; induced systemic resistance; plant-type hypersensitive response; systemic acquired resistance
MaB_DN1426_c0_g1	Ma06_p05860.1	plasma membrane	calcium ion binding	innate immune response; long-day photoperiodism; regulation of flower development; regulation of nitric oxide metabolic process; response to abscisic acid; response to absence of light;

				response to auxin; response to calcium ion; response to cold; response to heat; response to hydrogen peroxide; response to mechanical stimulus; response to metal ion
MaB_DN733_c0_g2	Ma08_p20080.1	.	.	.
MaB_DN1721_c0_g1	Ma01_p08390.1	chloroplast thylakoid membrane	copper ion binding; electron transfer activity	.
MaB_DN321_c0_g1	Ma07_p20600.1	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem II; plastoglobule	chlorophyll binding	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus
MaB_DN766_c1_g1	Ma10_p15370.1	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus
MaB_DN8953_c0_g2	Ma09_p30300.1	CCR4-NOT core complex; nucleus; P-body	3'-5' exonuclease activity; metal ion binding; poly(A)-specific ribonuclease activity; RNA binding	defense response to bacterium; exonucleolytic catabolism of deadenylated mRNA; negative regulation of translation; nuclear-transcribed mRNA poly(A) tail shortening
MaB_DN2736_c0_g1	Ma06_p26850.1	cytoplasm	metal ion binding; oxidoreductase activity acting on single donors with incorporation of molecular oxygen; incorporation of two atoms of oxygen	oxylipin biosynthetic process
MaB_DN1077_c0_g1	Ma06_p08680.2	chloroplast; chloroplast thylakoid membrane; integral component of membrane; photosystem I; thylakoid	.	photosynthesis; light harvesting in photosystem I; photosynthetic electron transport chain
MaB_DN344_c0_g1	Ma04_p39550.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding	photosynthesis, light harvesting; protein-chromophore linkage
MaB_DN7546_c0_g2	Ma09_p07910.1	.	carboxylic ester hydrolase activity; methyl indole-3-acetate esterase activity	pollen tube growth
MaB_DN57_c0_g1	Ma05_p10690.1	.	.	.
MaB_DN8953_c0_g1	Ma06_p20750.1	CCR4-NOT core complex; nucleus; P-body	3'-5' exonuclease activity; metal ion binding; poly(A)-specific ribonuclease activity; RNA binding	defense response to bacterium; exonucleolytic catabolism of deadenylated mRNA; negative regulation of translation; nuclear-transcribed mRNA poly(A) tail shortening
MaB_DN18653_c0_g2	NA	.	.	.
MaB_DN6653_c0_g2	Ma07_p08010.1	chloroplast thylakoid membrane; extrinsic component of membrane; photosystem II oxygen evolving complex	calcium ion binding	photosynthesis
MaB_DN489_c0_g2	Ma06_p16670.1	extracellular region	mannose binding	.
MaB_DN973_c1_g1	Ma03_p24340.1	cytoplasm; nucleus	.	.
MaB_DN8204_c1_g2	Ma04_p30440.1	apoplast, chloroplast stroma; chloroplast thylakoid membrane; photosystem II oxygen evolving complex; thylakoid lumen	calcium ion binding; electron transporter, transferring electrons within the cyclic electron transport pathway of photosynthesis activity	photosynthetic electron transport chain
MaB_DN1096_c0_g1	Ma04_p39550.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding	photosynthesis; light harvesting; protein-chromophore linkage
MaB_DN668_c0_g1	Ma08_p03860.1	chloroplast thylakoid membrane; photosystem II oxygen evolving	oxygen evolving activity	photosystem II assembly; photosystem II stabilization

		complex		
MaB_DN3084_c0_g1	Ma05_p16120.1	.	metal ion binding; ubiquitin-protein transferase activity	protein ubiquitination; response to cadmium ion; response to chitin
MaB_DN64878_c0_g1	Ma09_p24580.1	chloroplast thylakoid membrane	copper ion binding; electron transfer activity	.
MaB_DN475_c0_g3	Ma11_p01810.1	chloroplast	protochlorophyllide reductase activity	chlorophyll biosynthetic process; photosynthesis
MaB_DN3605_c0_g2	Ma09_p01450.2	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding	light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus
MaB_DN984_c0_g2	Ma04_p27610.1	chloroplast thylakoid membrane; integral component of membrane; photosystem II	.	photosynthesis; photosystem II stabilization
MaB_DN2188_c0_g1	Ma10_p12550.1	apoplast, chloroplast	glyceraldehyde-3-phosphate dehydrogenase (NAD+) (phosphorylating) activity; glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) activity; NAD binding; NADP binding	glucose metabolic process; reductive pentose-phosphate cycle; response to light stimulus
MaB_DN1185_c0_g1	Ma06_p14120.2	chloroplast; chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II antenna complex; plastoglobule; PSII associated light-harvesting complex II; thylakoid membrane	chlorophyll binding; metal ion binding; protein domain specific binding	nonphotochemical quenching; photosynthesis, light harvesting in photosystem I; photosystem II assembly; protein-chromophore linkage; response to blue light; response to far red light; response to light stimulus
MaB_DN984_c0_g1	Ma04_p27610.1	.	.	.
MaB_DN2931_c0_g1	NA	.	.	.
MaB_DN11395_c0_g1	Ma09_p09670.1	chloroplast; chloroplast envelope; chloroplast thylakoid; thylakoid membrane; integral component of membrane; photosystem I reaction center; plastoglobule	protein domain specific binding	photosynthesis
MaB_DN489_c0_g1	Ma06_p16670.1	extracellular region	mannose binding	.
MaB_DN531_c0_g1	Ma07_p10230.1	nuclear chromatin; nucleosome	DNA binding; protein heterodimerization activity	chromatin organization
MaB_DN317_c1_g1	Ma08_p15280.1	.	.	.
MaB_DN785_c0_g2	Ma08_p03640.1	chloroplast envelope; chloroplast thylakoid; thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage; response to blue light; response to cytokinin; response to far red light; response to light stimulus
MaB_DN730_c0_g1	Ma04_p32230.1	.	.	.
MaB_DN7850_c0_g1	Ma05_p03450.1	chloroplast	ATP binding; phosphoribulokinase activity	reductive pentose-phosphate cycle
MaB_DN11003_c0_g1	Ma08_p12270.1	.	.	.
MaB_DN6975_c0_g4	Ma09_p22990.1	.	.	.
MaB_DN344_c0_g2	Ma10_p15370.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding	photosynthesis, light harvesting; protein-chromophore linkage
MaB_DN7316_c1_g1	Ma08_p17130.1	.	.	.
MaB_DN16544_c0_g2	Ma06_p06320.3	.	.	.
MaB_DN8805_c0_g1	Ma08_p30830.1	nucleus; protein-containing complex	protein homodimerization activity; zinc ion binding	response to water deprivation

MaB_DN13574_c0_g1	Ma02_p01750.1	nucleus	calmodulin binding	regulation of salicylic acid metabolic process; response to salt stress; response to water deprivation
MaB_DN1150_c0_g3	Ma07_p06960.1	nucleus	DNA binding; DNA-binding transcription factor activity	defense response; ethylene-activated signaling pathway
MaB_DN6653_c0_g1	Ma07_p08010.1	chloroplast thylakoid membrane; extrinsic component of membrane; photosystem II oxygen evolving complex	calcium ion binding	photosynthesis
MaB_DN3888_c0_g1	Ma05_p11320.1	.	.	.
MaB_DN936_c1_g1	Ma08_p33260.1	amyloplast	calcium ion binding; chitinase activity	chitin catabolic process; polysaccharide catabolic process; seed germination; seedling development
MaB_DN1416_c1_g3	Ma04_p21170.1	nucleus	DNA-binding transcription factor activity	defense response; ethylene-activated signaling pathway
MaB_DN1061_c0_g2	Ma09_p09900.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I	.	photosynthesis
MaB_DN16455_c0_g1	Ma07_p09650.1	.	.	.
MaB_DN32471_c0_g1	Ma05_p26840.1	.	.	.
MaB_DN9613_c0_g1	Ma09_p22170.1	.	.	.
MaB_DN344_c1_g1	Ma02_p11170.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding	photosynthesis; light harvesting; protein-chromophore linkage
MaB_DN770_c1_g1	NA	.	.	.
MaB_DN7091_c0_g1	Ma11_p20650.1	chloroplast	glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating) activity; NAD binding; NADP binding	glucose metabolic process; reductive pentose-phosphate cycle
MaB_DN9475_c0_g1	Ma02_p16400.1	.	lipid binding	lipid transport
MaB_DN6765_c0_g2	Ma02_p11170.1	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding	photosynthesis, light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus
MaB_DN5894_c0_g2	Ma09_p21570.1	chloroplast; chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding; protein domain specific binding; structural molecule activity	photosynthesis, light harvesting in photosystem I; light harvesting in photosystem II; protein-chromophore linkage; regulation of stomatal movement; response to abscisic acid; response to herbicide; response to high light intensity: response to light stimulus
MaB_DN1419_c0_g2	Ma04_p17650.1	chloroplast thylakoid membrane; photosystem I reaction center	.	photosynthesis
MaB_DN23730_c1_g1	Ma03_p03390.1	.	heme binding; metal ion binding; peroxidase activity	hydrogen peroxide catabolic process; response to oxidative stress
MaB_DN92726_c0_g1	Ma04_p31600.1	integral component of membrane; plasma membrane	ATP binding; protein serine/threonine kinase activity	.
MaB_DN344_c0_g4	Ma04_p39550.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding	photosynthesis; light harvesting; protein-chromophore linkage
MaB_DN17080_c0_g2	Ma09_p27170.1	nucleus	DNA binding; metal ion binding	.
MaB_DN33262_c0_g1	NA	.	.	.
MaB_DN5666_c0_g2	Ma06_p22130.1	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane;	chlorophyll binding; metal ion binding	photosynthesis, light harvesting in photosystem I; protein-chromophore

		photosystem I; photosystem II; plastoglobule		linkage; response to light stimulus
MaB_DN167_c0_g3	Ma04_p17220.1	.	lipid binding	lipid transport
MaB_DN344_c0_g5	Ma10_p15370.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding	photosynthesis, light harvesting; protein-chromophore linkage
MaB_DN11415_c0_g1	Ma07_p06000.1	nucleus	DNA-binding transcription factor activity; sequence-specific DNA binding	abscisic acid-activated signaling pathway; glucosinolate metabolic process
MaB_DN18785_c0_g1	Ma05_p26790.2	.	.	.
MaB_DN1776_c0_g1	NA	.	.	.
MaB_DN3328_c0_g1	Ma03_p07810.1	.	.	.
MaB_DN8109_c0_g3	Ma07_p28730.1	mitochondrion	glycine hydroxy methyl-transferase activity; pyridoxal phosphate binding	glycine biosynthetic process from serine; tetrahydrofolate interconversion
MaB_DN1217_c0_g1	Ma06_p08680.2	chloroplast; chloroplast thylakoid membrane; integral component of membrane; photosystem I; thylakoid	.	photosynthesis; light harvesting in photosystem I; photosynthetic electron transport chain
MaB_DN5274_c0_g1	Ma00_p03960.1	.	magnesium ion binding; terpene synthase activity	.
MaB_DN129_c0_g2	Ma08_p33270.1	vacuole	chitinase activity; lysozyme activity	chitin catabolic process; polysaccharide catabolic process
MaB_DN9720_c0_g1	Ma01_p20450.1	.	.	.
MaB_DN1419_c0_g1	Ma05_p14680.1	chloroplast thylakoid membrane; photosystem I reaction center	.	photosynthesis
MaB_DN2315_c1_g2	Ma06_p26850.1	cytoplasm	metal ion binding; oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	oxylipin biosynthetic process
MaB_DN4486_c0_g2	Ma06_p30010.1	chloroplast thylakoid membrane; integral component of membrane; photosystem II	manganese ion binding	photosynthesis
MaB_DN2915_c0_g1	NA	.	.	.
MaB_DN1671_c0_g1	Ma00_p03960.1	.	magnesium ion binding; terpene synthase activity	.
MaB_DN595_c0_g1	Ma10_p05350.1	cytosol; plant-type cell wall; plasmodesma	heme binding; metal ion binding; peroxidase activity	defense response to fungus; hydrogen peroxide catabolic process; response to oxidative stress; rhythmic process
MaB_DN4968_c0_g1	Ma04_p32230.1	cell wall; extracellular region	.	plant-type cell wall organization
MaB_DN1274_c1_g1	Ma06_p38350.1	integral component of membrane; vacuolar membrane	pyrophosphate hydrolysis-driven proton transmembrane transporter activity; inorganic diphosphatase activity; metal ion binding	proton transmembrane transport
MaB_DN16140_c0_g1	Ma04_p14940.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding	photosynthesis, light harvesting; protein-chromophore linkage
MaB_DN668_c0_g3	Ma08_p03860.1	chloroplast thylakoid membrane; photosystem II oxygen evolving complex	oxygen evolving activity	photosystem II assembly; photosystem II stabilization
MaB_DN4486_c1_g1	Ma08_p29270.1	chloroplast thylakoid membrane; integral component of membrane; photosystem II	manganese ion binding	photosynthesis
MaB_DN2687_c1_g1	Ma05_p17280.1	extracellular region	hydrolase activity; acting on	cuticle development; lipid catabolic

			ester bonds	process
MaB_DN15560_c0_g3	Ma09_p06640.1	chloroplast envelope; chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II; plastoglobule	chlorophyll binding; metal ion binding	photosynthesis; light harvesting in photosystem I; protein-chromophore linkage; response to light stimulus
MaB_DN10592_c0_g5	NA	.	.	.
MaB_DN9417_c0_g1	Ma03_p08980.1	chloroplast	ATP binding; glutamate-ammonia ligase activity	glutamine biosynthetic process
MaB_DN67987_c0_g1	Ma04_p23780.1	.	calcium ion binding	trichome branching
MaB_DN79356_c0_g1	Ma06_p18330.1	.	.	.
MaB_DN8358_c2_g1	Ma09_p07130.1	cytoplasm; cytosol; nuclear envelope; nucleus	Ran GTPase binding	miRNA loading onto RISC involved in gene silencing by miRNA; protein import into nucleus
MaB_DN11059_c0_g1	NA	.	.	.
MaB_DN17024_c0_g1	Ma02_p11170.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I; photosystem II	chlorophyll binding; metal ion binding	photosynthesis; light harvesting; protein-chromophore linkage
MaB_DN16139_c0_g1	Ma05_p30610.1	.	DNA-binding transcription factor activity; metal ion binding	regulation of transcription, DNA-templated
MaB_DN4631_c0_g2	Ma01_p10240.1	chloroplast; chloroplast thylakoid; thylakoid membrane; integral component of membrane; photosystem II, PSII associated light-harvesting complex II	chlorophyll binding; protein domain specific binding; xanthophyll binding	nonphotochemical quenching; photosynthesis; response to karrikin; thylakoid membrane organization
MaB_DN5430_c0_g1	Ma02_p11040.1	chloroplast thylakoid membrane; integral component of membrane; photosystem I reaction center	.	photosynthesis