J. Trop. Agric. and Fd. Sc. 44(1)(2016): 121 – 136

Digital gene expression of water-stressed *Phyllanthus urinaria* (Dukung anak)

V. Maheswary[1], H.N. Khairun[2], S. Vasanthi[2]and O. Nur Qistina[2]

[1]Strategic Planning & Innovation Management Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia
[2]Biotechnology and Nanotechnology Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

Abstract
In this study, we investigated the up- and down-regulation of genes involved in the secondary metabolite biosynthesis pathways in mature dukung anak (*Phyllanthus urinaria*) plants known to produce phytochemicals. Phytochemicals could contribute to human health by subjecting them to drought stress under net house conditions. Our raw data analyses using digital gene expression (DGE) technology showed that the number of sequence tags in the water-stressed plants was 1.9 folds more than in the control plants. Blast analysis showed that more than 90% of these sequences matched to the annotated sequences in the plantTA database with an up- or down-regulation of several genes in the main isoprenoid, shikimate, fatty acids, steroids and amino acids biosynthesis pathways in the water-stressed plants. Several enzymes were induced after water-stress but at a low frequency while some were either completely suppressed or showed no change in their expression. Gene Ontology (GO) analysis at level 2 revealed a significantly higher number of annotated sequences (E-value $\leq 10^{-6}$) in the water-stressed plants in each of the biological process, molecular function and cellular component categories, compared to the control plants. The results clearly indicated the beneficial effect of water-stress in increasing the production of several useful secondary metabolites in *P. urinaria* plants.

Keywords: differential gene expression, secondary metabolites biosynthesis pathways, phytochemicals, gene ontology

Introduction

Plants produce a wide variety of secondary metabolites or phytochemicals that not only help to protect the plant in various ways, but are also known to contribute to human health by reducing and preventing the risk of certain diseases. In addition, they also play an important role in human nutrition. The genus *Phyllanthus* belonging to the family Euphorbiaceae, is one such plant that has been widely used as a traditional medicinal plant in many tropical countries and reported to produce these phytochemicals including polyphenols, benzenoids, coumarins, esters, flavonoids, sterols, alkaloids, triterpenes, terpenoids, lactones, steroids, lignans and tannins (Satyan et al. 1995; Chang et al. 2003; Wang and Lee 2005). These compounds have been proven to have potential beneficial therapeutic

Article history
Received: 26.9.2012
Accepted: 9.9.2015

Authors' full names: Maheswary Vellupillai, Khairun Hisam Nasir, Vasanthi Sivarajah and Nur Qistina Othman
E-mail: mahes@mardi.gov.my

actions such as antiviral, antibacterial, antifungal, antitumour, hepatoprotective, antihypertensive and antidiabetic properties (Calixto et al. 1998; Yang et al. 2005; Huang et al. 2006).

Several compounds have already been isolated and identified from *Phyllanthus urinaria* L., an annual herb, locally known as dukung anak. These include corilagin, rutin, gallic acid, ellagic acid, geraniin, 1,3,4,6-tetra-*O*-galloyl-β-D-glucose and germin D. Geraniin and 1,3,4,6-tetra-*O*-galloyl-β-D-glucose, both extracted from the acetone extract of this fresh whole plant have been shown to actively inhibit herpes simplex virus-2 (HSV-2) and HSV-1 infections *in vitro* respectively (Yang et al. 2007). Ellagitannin present in this plant has been proven to have strong antiviral activity against Epstein-Barr virus DNA polymerase (Liu et al. 1999). Other *Phyllanthus* species such as *P. amarus* have also been shown to have potent antioxidant activity in addition to anti-inflammatory, antimutagenic, anticarcinogenic and antidiarrhoeal activities (Modak et al. 2007).

Biochemical pathways leading to secondary product accumulation in plants have been shown to be triggered and turned on by various biotic and abiotic stresses such as low temperature, ultra violet (UV) radiation, heavy metals, high-light illumination, wounding, drought, low nutrients and pathogen attack (Yamasaki et al. 1995; Laila 2006; Sharma and Dietz 2006; Indrajith and Ravindran 2009). These stress conditions generate reactive oxygen species (ROS) such as O_2, H_2O_2, OH and O_2, extra- or intra-cellularly. These are toxic to plant cells and cause oxidative damage to nucleic acids, lipids and proteins that may make them non-functional.

However, plants are capable of adapting to these environmental changes by adjusting their gene expression levels, often at the transcription initiation stage to activate a wide range of defence mechanisms that function to increase tolerance to the limiting conditions. Their early responses are the stress signal perception and subsequent signal transduction which lead to the activation of various molecular, biochemical and physiological responses (Yamaguchi and Shinozaki 2006).

Plant cells are equipped with excellent antioxidant defense mechanisms which could be either non-enzymatic or enzymatic to detoxify the harmful effects (Sharma and Dietz 2006; Ahmad et al. 2009; Gill and Tuteja 2010). The enzymatic antioxidant defense mechanisms include enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), guaicol peroxidase (GOPX), glutathione-S-transferase (GST) and glutathione reductase (GR).

Plant phenolic compounds such as flavonoids and lignin precursors, also known as dietary phytophenolics, have also been recognised largely as beneficial antioxidants that can scavenge harmful ROS. They belong to the non-enzymatic antioxidant defence mechanism and are important constituents of the human diet (Indrajith and Ravindran 2009). These flavonoid compounds are also capable of acting as a UV filter, thus protecting the photosynthetic tissues from damage (Liang et al. 2006). Other non-enzymatic antioxidant defence mechanisms include ascorbic acid (ASH), glutathione (GSH), proline, alkaloids, non-protein amino acids and alpha-tocopherols.

Some isoprenoids such as carotenoids and tocopherols have also been reported to play an effective role in plant metabolism including oxidative stress tolerance and photoprotection (Penuelas and Munne-Bosch 2005). Vitamin C (ascorbate) functions as an ubiquitous antioxidant in both animals and plants by scavenging ROS via both the enzymatic and non-enzymatic reactions (Sakihama et al. 2002).

With the availability of genomic sequences from various plant species including both model plants such as

Arabidopsis (Matsui et al. 2008) and crops such as rice (*Oryza sativa*) (Zhou et al. 2007) and soybean (Le et al. 2012), genes associated with drought/dehydration responses have already been identified. In this study, we performed digital gene expression (DGE) analysis to identify these genes in *P. urinaria* under water-stress conditions. It is a high-throughput, robust and sensitive sequencing technology (Shendure and Ji 2008) for detection and comparison of expression profiles between treated and non-treated samples (Veitch et al. 2010). It can analyse hundreds of millions of DNA fragments simultaneously, generating giga-bases of sequence information from a single run, thus generating a digital output proportional to the number of transcripts per mRNA. This technology has made a huge impact on genomic research because of the depth and unbiased coverage of the entire transcriptome.

Materials and methods

Phyllanthus urinaria plants were grown under net house conditions at MARDI, Serdang. When the plants reached maturity after about 2 months and the height of the plants were about 30 cm, one half of the plants were subjected to water-stress treatment for 5 days continuously by irrigating with an average of 250 ml water per day, while the other half was irrigated normally. A total of 5 g portions of the stems and leaves were harvested (*Figure 1*), wrapped in aluminium foil and snap frozen in liquid nitrogen before storage at –80 °C until further use.

RNA isolation

Total RNA was isolated from the frozen stems and leaves of both control and water-stressed samples by grinding to a fine powder in a mixture mill using liquid N_2. Cetyltrimethylammonium bromide (CTAB) reagent was then added to the ground material and subsequent steps were performed according to the method described by Chang et al. (1993). The

Figure 1. ***Phyllanthus urinaria*** *plants under net house condition a) Water-stressed; b) Control plants*

total RNA was resuspended in RNase-free water and the RNA aliquots were quantified on a ND-1000 nanodrop (Nano Drop Technologies Inc, USA). Gel-electrophoresis was performed on a 1% formaldehyde agarose gel to check the integrity of the RNA extracted. The RNA was then purified using the RNeasy Plant Mini Kit (Qiagen, Germany) and the quantity and quality of the purified RNA aliquots were checked again using the above methods. A total of 20 µg of purified total RNA (500 ng/µl) from each of the control and water-stressed samples were outsourced to Illumina company (USA) to generate two DGE libraries.

Digital gene expression

Digital gene expression was carried out using the second Illumina Genome Analyser platform (GA II) based on the addition of specific adapters to poly(A) cDNA, which has been digested with a restriction enzyme, typically NlaIII. Further digestion of the cDNA with another enzyme (Mme I) that recognises within the adapter but cleaves 21-bp downstream, generates a 21-base tag from that transcript. These tags are directly sequenced using next generation sequencing technology, allowing direct quantification of the number of transcripts in a sample. This process will generate sequence tags from transcripts that contain NlaIII sites, preferentially from the 3' end of the transcripts in the untranslated regions (UTRs) or the open reading frame (ORF).

BLAST analysis and identification of DGE sequence tags

The 21-bp sequence tags from the control and water-stressed DGE libraries were matched against the TIGR Plant Transcript Assembly (plantTA) database (http://plantta.tigr.org/) (Childs et al. 2007) using the Basic Local Alignment Sequencing Tool (BLAST) program. This database uses expressed sequences collected from the NCBI Gene Bank Nucleotide database for the construction of transcript assemblies. The database includes all plant species for which more than 1,000 Expressed Sequence Tags (ESTs) and full-length and partial cDNA sequences are publicly available. The best hit out of ten hits was then selected for each sequence tag based on genes identified to be associated with secondary metabolites biosynthesis pathways or with the highest scoring pair of nucleotides.

Functional analysis using Gene ontology (GO)

PlantTA sequences that mapped to the 21-bp sequence tags were blast against SwissProt database with a cut-off E-value of $\leq 10^{-6}$ before performing functional analysis on the resulting peptide sequences using the Blast2GO program (http://www.blast2go.de/) (Conesa et al. 2005; Gotz et al. 2008). Sequences were annotated according to the three main functional categories of Biological Process (BP), Molecular Function (MF) and Cellular Component (CC) (Ashburner et al. 2000; Botton et al. 2008). These groups can be queried at different levels depending on the depth of knowledge and specificity about that entity (Gene Ontology Consortium 2006; Gotz et al. 2008).

Results and discussion

Raw sequence analysis of DGE sequence tags

A total of about 5 million 21-bp raw sequence tags were generated by single pass (5'-sense strand) sequencing from each DGE library. The total number of sequence tags in the control and water-stressed libraries with frequencies of 1, 2 and ≥3 was 444,509 and 431,504 respectively. After elimination of sequence tags with frequencies ≤2 (poor quality sequencing scores due to sequencing error, giving rise to runs of the same base artefacts), the total number of sequence tags with frequencies of ≥3 remaining in the control and water-stressed libraries for blast analysis were 156,313 and 293,160 respectively.

Blast analysis of DGE sequence tags

After Blast analysis, results indicated that over 90% (143,320 in the control and 285,127 in the water-stressed) of the raw sequence tags with frequencies of ≥3 matched to the annotated sequences in the plantTA database. A total of 6,822 and 7,769 sequence tags from the control and water-stressed libraries respectively, showed no match to the sequences in the plantTA database. An additional 6,171 and 264 raw sequences from the control and water-stressed libraries respectively, were missing after blast analysis. These sequence tags could have been recognised by the program as possible artefacts (runs of the same base), and therefore omitted from analysis (Philippe et al. 2013).

Identification of differentially regulated genes

- **Isoprenoid biosynthesis pathway**
  We identified two enzymes from the main isoprenoid biosynthesis pathway, tocopherol cyclase (VTE1) and gamma tocopherol methyltransferase (γ-TMT) which were up-regulated 1.9 and 1.5 folds respectively, as shown in *Table 1*.

  In the terpenoid backbone biosynthesis pathway, citrate (Si) synthase was up-regulated 3.2 folds while there was no change in the expression level of 1-deoxy-D-xylulose-5-phosphate synthase after water-stress. However, isopentenyl-diphosphate Delta-isomerase was down-regulated 1.6 folds.

Table 1. Differential expression of putative genes identified from the isoprenoid biosynthesis pathway leading to carotenoid, monoterpenoid and sesquiterpenoid biosynthesis in *Phyllanthus urinaria* under water-stress

Biosynthesis pathways and putative genes identified (including isoforms)	Frequency of 21-bp sequence tags		Fold increase/ decrease	Biosynthesis pathways and putative genes identified (including isoforms)	Frequency of 21-bp sequence tags		Fold increase/ decrease
	Control	Water-stress			Control	Water-stress	
Carotenoid biosynthesis				**Isoprenoid biosynthesis**			
Phytoene synthase (PSY) [2.5.1.32]*	426	763	1.8	Tocopherol cyclase (VTE1) [–]	103	195	1.9
Phytoene desaturase (PDS) [–]	182	180	1.0	Gamma tocopherolmethyltransferase (γ-TMT) [–]	151	233	1.5
Zeaxanthin epoxidase (ZEP) [1.14.13.90]	151	169	1.1				
Carotenoid isomerase (CRTISO) [–]	66	108	1.6				
Violaxanthin de-epoxidase (VDE) [1.10.99.3]	57	156	2.7				
Lycopene beta cyclase (β-LCY) [–]	–	21	–	**Diterpenoid biosynthesis**			
Neoxanthin synthase (NSY) [–]	–	4	–	Copalyl diphosphate synthase	8	12	1.5
Capsanthin/Capsorubin synthase [5.3.99.8]	–	20	–				
9-cis-epoxycarotenoid dioxygenase (NCED) [1.13.11.51]	915						
Beta-carotene hydroxylase (CHX) [–]		467	2.0	**Terpenoid backbone biosynthesis**			
Epsilon lycopene cyclase (ζ-LYC) [–]	497	475	0.9	1-deoxy-D-xylulose-5-phosphate synthase [2.2.1.7]	231	231	–
Carotenoid cleavage dioxygenase [–]	129	82	1.6				
Carotenoid oxygenase [–]	397	328	1.2	Isospentenyl-diphosphate Delta-isomerase [5.3.3.2]	57	36	1.6
	6	–	–				
				Citrate (Si)-synthase [2.3.3.1]	13	42	3.2
Monoterpenoid biosynthesis				**Sesquiterpenoid biosynthesis**			
(+)-neomenthol dehydrogenase [1.1.1.208]	–	23	–	(+)-delta-cadinene synthase [4.2.3.13]	34	57	1.7
Linalool synthase [–]	476	522	1.1	(-)-germacrene D synthase [–]	388	277	1.4
Myrcene synthase [4.2.3.15]	24	36	1.5	(+)-delta-cadinene synthase [4.2.3.13]	34	57	1.7
Pinene synthase [4.2.3.14]	17	22	1.3	Aristolochene synthase [4.2.3.9]	613	101	6.1
				Farnesyl pyrophosphate synthetase (FPPsynthetase) [–]	479	203	2.4

*Enzyme commission numbers in [ ] in secondary metabolite biosynthesis pathways in KEGG database

A total of four enzymes were also up-regulated in the carotenoid biosynthesis pathway including phytoene synthase (PSY) (1.8 folds), carotenoid isomerase (CRTISO) (1.6 folds), zeaxanthin epoxidase (ZEP) (1.1 folds) and violaxanthin de-epoxidase (VDE) (2.7 folds) (*Table 1*). A total of three enzymes were induced after water-stress treatment including lycopene beta cyclase (β-LCY), neoxanthin synthase (NSY) and capsanthin/capsorubin synthase. This increase in the synthesis of carotenoids may play a part in oxidative stress tolerance and photoprotection as described by Penuelas and Munne-Bosch (2005). However, four other transcripts were down-regulated in this pathway, including 9-cis-epoxycarotenoid dioxygenase (NCED) (2.0 folds), beta-carotene hydroxylase (CHX) (0.9 folds), epsilon lycopene cyclase (ζ-LCY) (1.6 folds) and carotenoid cleavage dioxygenase (1.2 folds). In addition, carotenoid oxygenase was totally suppressed after water-stress treatment while there was no change in the expression of phytoene desaturase.

In the monoterpenoid biosynthesis pathway, three enzymes including myrcene synthase, pinene synthase and linalool synthase were up-regulated 1.1 – 1.5 folds (*Table 1*) while (+)-neomenthol dehydrogenase was induced after water- stress.

In the sesquiterpenoid biosynthesis pathway, (–)-germacrene D synthase, aristolochene synthase and farnesyl pyrophosphate (FPP) synthetase were down-regulated 1.4, 6.1 and 2.4 folds respectively, while (+)-delta-cadinene synthase was up-regulated 1.7 folds. In the diterpenoid biosynthesis pathway, only one enzyme, copalyl diphosphate synthase, was up-regulated 1.5 folds. Yadav et al. (2013) also found some minor monoterpenes, all sesquiterpenes and other low molecular weight volatiles of essential oil components induced by water deficit treatment in *Artemisia annua* L.

- **Shikimate biosynthesis pathway**

Table 2 shows the differential expression of enzymes in the shikimate biosynthesis pathway leading to phenylpropanoid, flavonoid, isoflavonoid, anthocyanin and flavone and flavonol biosynthesis after water-stress treatment.

A total of 12 enzymes in the phenylpropanoid biosynthesis pathway showed increased expression after water stress including trans-cinnamate 4-monooxygenase(1.9 folds), trans-cinnamate 4-hydroxylase (C4H) (1.5 folds), transketolase (1.3 folds), transaldolase (1.2 folds), Class III peroxidase (1.8 folds), caffeate O-methyltransferase (COMT-1)(3.3 folds), cinnamoyl CoA reductase (1.1 folds), cinnamyl-alcohol dehydrogenase (1.4 folds), shikimate dehydrogenase (2.4 folds), 6-phosphofructokinase (6.4 folds), dihydrolipoyl dehydrogenase (4.9 folds) and glutathione peroxidase (1.3 folds) (*Table 2*). This was in agreement with the findings of Dixon and Paiva (1995) who also reported that phenylpropanoid metabolism increased in response to many abiotic stresses such as high light/UV, low temperature, low phosphate and drought stress. However, seven of the enzymes in this pathway were also down-regulated after water-stress treatment including ferulate 5-hydroxylase (F5H) (2.3 folds), phenylalanine ammonia-lyase (PAL) (0.05 fold), caffeoyl-CoA O-methyltransferase (1.4 folds), coumarate 3-hydroxylase (3.9 folds), caffeic acid O-/3-O-methyltransferase (1.7 folds), alpha/beta-D-glucosidase (2.3 folds) and 3-phosphoshikimate 1-carboxyvinyltransferase (1.3 folds). The expression of 4-Coumarate CoA ligase was induced after water-stress

Table 2. Differential expression of putative genes identified from the shikimate biosynthesis pathway leading to the phenylpropanoid, flavonoid, isoflavonoid, anthocyanin and flavone and flavonol biosynthesis in *Phyllanthus urinaria* under water-stress

Biosynthesis pathways and putative genes identified (including isoforms)	Frequency of 21-bp sequence tags		Fold increase/ decrease	Biosynthesis pathways and putative genes identified (including isoforms)	Frequency of 21-bp sequence tags		Fold increase/ decrease
	Control	Water-stress			Control	Water-stress	
Phenylpropanoid biosynthesis				**Flavonoid/Stillbene biosynthesis**			
Transaldolase[2.2.1.2]*	1,455	1,815	1.2	Resveratrol [–]	24	40	1.7
Caffeate O-methyltransferase (COMT-1) [2.1.1.68]	50	164	3.3	Leucoanthocyanidin reductase (LAR) [1.17.1.3]	2,832	3,007	1.1
Cinnamyl-alcohol dehydrogenase [1.1.1.195]	249	341	1.4	Chalcone isomerase (CHI) [5.5.1.6]	1,676	1,326	1.3
4-Coumarate CoA ligase [6.2.1.12]	–	18	–	Chalcone synthase (CHS) [–]	7,321	5,972	1.2
Trans/Cinnamate 4-hydroxylase (C4H) [–]	377	574	1.5	Chalconeand Stillbene synthases [–]	989	912	1.1
Transketolase [2.2.1.1]	417	551	1.3	Chalcone reductase [–]	78	63	1.2
Trans-cinnamate 4-monooxygenase [1.14.13.11]	186	361	1.9	Chalcone-flavonone isomerase [–]	243	37	6.6
Shikimate dehydrogenase [1.1.125]	58	137	2.4	Dihydroflavonol 4-reductase (DFR) [–]	1,381	1,020	1.4
Cinnamoyl CoA reductase [–]	874	991	1.1	Naringenin-chalcone synthase [2.3.1.74]	32	18	1.8
Class III peroxidase [–]	49	86	1.8	Leucoanthocyanidin dioxygenase [–]	71,046	17,100	4.2
6-phosphofructokinase [2.7.1.11]	23	148	6.4	Flavonol synthase/flavanone 3-hydroxylase (F3H) [1.14.11.23]	2,362	1,566	1.5
Dihydrolipoyl dehydrogenase [1.8.1.4]	11	54	4.9				
Glutatathione peroxidase (GPX) [–]	562	706	1.3				
Coumarate-3-hydroxylase	82	21	3.9	**Isoflavonoid biosynthesis**			2
Ferulate 5-hydroxylase (F5H) [–]	1,491	641	2.3	Isoflavone 2'-hydroxylase [1.14.13.89]	4	9	.3
Phenylalanine ammonia-lyase (PAL) [–]	9,571	9,065	0.1	Isoflavone reductase [–]	74	89	1.2
Caffeoyl-CoA O-methyltransferase [2.1.1.104]	1,907	1,371	1.4	Isoflavone synthase [–]	210	205	1.0
Caffeic acid O-/3-O-methyltransferase [–]	15,785	9,046	1.7	Isoflavone 7-O-methyltransferase [–]	4,067	285	14.3
Alpha/Beta-D-glucosidase [3.2.1.21]	877	377	2.3				
3-phosphoshikimate 1-carboxyvinyltransferase [2.5.1.19]	227	176	1.3	**Flavone and flavonol biosynthesis**			
				Flavonoid 3'-monooxygenase [–]	7	7	–
Caffeine synthase [–]	4	4	–	Flavonoid 3',5'-hydroxylase [1.14.13.88]	922	89	1.0
				Anthocyanin biosynthesis			
				Anthocyanidin synthase [–]	255	452	1.8
				Anthocyanidin 3-O- glucosyltransferase [2.4.1.115]	714	920	1.3
				Anthocyanidinerhamnosyl-transferase [–]	4	36	9.0
				Anthocyanidin reductase (ANR) [–]	313	345	1.1

*Enzyme commission numbers in [ ] in secondary metabolite biosynthesis pathways in KEGG database

while there was no change in the expression of caffeine synthase.

In the flavonoid/stillbene biosynthesis pathway, we identified two enzymes, leucoanthocyanidin reductase (LAR) and resveratrol which were up-regulated 1.1 and 1.7 folds respectively, after water-stress (*Table 2*). Enzymes in this pathway have previously been shown to have antioxidant activity against a variety of oxidisable compounds (Polovka et al. 2003). Flavonoid compounds produced by these enzymes are recognised as beneficial antioxidants and are important constituents of the human diet as described by Indrajith and Ravindran (2009). However, nine other enzymes identified including chalcone isomerase (CHI), chalcone synthase (CHS), chalcone and stillbene synthases, chalcone reductase, chalcone-flavonone isomerase, dihydroflavonol 4-reductase (DFR), naringenin-chalcone synthase, leucoanthocyanidin dioxygenase and flavonol synthase/ flavanone 3-hydroxylase (F3H) were all down-regulated between 1.1 and 6.6 folds (*Table 2*).

In the isoflavonoid biosynthesis pathway, isoflavone 2'-hydroxylase and isoflavone reductase were up-regulated 2.3 and 1.2 folds respectively, under water-stress conditions while isoflavone synthase and isoflavone 7-O-methyltransferase were down-regulated 1.0 and 14.3 folds respectively.

In the flavone and flavonol biosynthesis pathway, there was no change in the expression of flavonoid 3'-monooxygenase while flavonoid 3'5'-hydroxylase was down-regulated 1.0 fold.

A total of four enzymes identified in the anthocyanin biosynthesis pathway, including anthocyanidin 3-O-glucosyltransferase, anthocyanidin synthase, anthocyanidine rhamnosyl-transferase and anthocyanidin reductase were up-regulated 1.3, 1.8, 9.0 and 1.1 folds respectively. One of the proposed roles of anthocyanins is amelioration of stress damage due to antioxidant activity. Anthocyanin biosynthetic pathway genes such as glycosyltransferases and anthocyanidin rhamnosyl-transferase have been shown to be stress-induced in plants such as horsegram (Chalker-Scott 1999; Reddy et al. 2008).

- **Fatty acids biosynthesis pathway**
Table 3 shows the differential expression of enzymes identified in the fatty acid biosynthesis pathway leading to biosynthesis of saturated and unsaturated fatty acids, steroids and glycosphingolipids after water-stress treatment.

In the saturated fatty acids biosynthesis pathway, a total of four enzymes were identified to be up-regulated 1.2 – 1.5 folds including acetyl-CoA carboxylase, biotin carboxylase, orcinol O-methyltransferase and omega-3/6 fatty acid desaturase. There was no change in the expression of 3-ketoacyl-CoA thiolase while 3-ketoacyl-CoA reductase was down-regulated 1.3 folds. The expression of acetyl Co-A synthetase was completely suppressed after water-stress.

Meanwhile, in the unsaturated fatty acids pathway, both acyl-CoA oxidase and enoyl-CoA hydratase were up-regulated 1.1 and 1.0 folds respectively, while acyl-[acyl-carrier-protein-] desaturase was down-regulated 1.6 folds. Saturated and unsaturated fatty acids found in plants are known to have beneficial effects on health.

In the glycosphingolipid biosynthesis series, the expression of galactoside 2-alpha-L-fucosyltransferase was up-regulated 1.8 folds while alpha/beta galactosidase was down-regulated 1.1 folds.

A total of seven enzymes identified in the steroid biosynthesis pathway were up-regulated 1.2 – 4.2 folds (*Table 3*)

Table 3. Differential expression of putative genes identified from the fatty acids biosynthesis pathway leading to unsaturated fatty acids, steroids and glycosphingolipids biosynthesis in *Phyllanthus urinaria* under water-stress

Biosynthesis pathways and putative genes identified (including isoforms)	Frequency of 21-bp sequence tags		Fold increase/ decrease	Biosynthesis pathways and putative genes identified (including isoforms)	Frequency of 21-bp sequence tags		Fold increase/ decrease
	Control	Water-stress			Control	Water-stress	
Saturated fatty acidsbiosynthesis				**Steroid biosynthesis**			
Acetyl-CoA carboxylase [6.4.1.2]*	434	599	1.4	Squalene monooxygenase [1.14.99.7]	1,155	4,468	3.9
Biotin carboxylase [6.3.4.14]	425	641	1.5	Squalene synthase [2.5.1.21]	425	625	1.5
Orcinol O-methyltransferase [–]	226	263	1.2	Sterol-8,7-isomerase [–]	84	157	1.9
Omega-3/6 fatty acid desaturase [–]	444	576	1.3	Methylenesterol C-methyltransferase [–]	805	1,156	1.4
Acetyl-CoA synthetase[–]	58	–	–	C-4 sterol methyl oxidase [–]	10	42	4.2
3-ketoacyl-CoA reductase [–]	602	455	1.3	Steroid 23-alpha-hydroxylase [–]	32	37	1.2
3-ketoacyl-CoA thiolase [–]	9	9	–	Sterol 4-alpha-methyl-oxidase [–]	–	44	–
				3-beta-hydroxy/-delta5-steroid dehydrogenase [–]	959	2,800	2.9
Unsaturated fatty acids biosynthesis							
Enoyl-CoA hydratase [4.2.1.17]	470	487	1.0	Sterol 24-C-methyltransferase [2.1.1.41]	–	11	–
Acyl-CoA oxidase [1.3.3.6]	222	241	1.1	Sterol delta-7 reductase (DWF5) [–]	239	147	1.6
Acyl-[acyl-carrier-protein] desaturase [1.14.19.2.]	11	7	1.6				
Glycosphingolipid biosynthesis							
Galactoside 2-alpha-L-fucosyltransferase_ [2.4.1.69]	20	35	1.8				
Alpha/Beta-galactosidase [3.2.1.22/3]	840	736	1.1				

*Enzyme commission numbers in [ ] in secondary metabolite biosynthesis pathways in KEGG database

including squalene monooxygenase, methylenesterol *C*-methyltransferase, 3-beta-hydroxy/-delta5-steroid dehydrogenase, squalene synthase, C-4 sterol methyl oxidase, steroid 23-alpha-hydroxylase and sterol-8,7-isomerase. Interestingly, two enzymes, sterol 24-C-methyltransferase and sterol 4-alpha-methyl-oxidase were induced after water-stress. Enzymes involved in steroid biosynthesis produce bioactive compounds or sterols such as β-sitosterol, campestrol and stigmasterol which are known to have properties that can reduce the absorption of dietary cholesterol from the gut as well as help in assisting the cholesterol elimination from the body. Only one enzyme, sterol delta-7 reductase (DWF5) was down-regulated (1.6 folds).

- **Amino acids biosynthesis pathways**
  In the phenylalanine, tyrosine and tryptophan biosynthesis pathway (*Table 4*), 3 enzymes, namely, chorismate synthase, tryptophan synthase and CAT were up-regulated 1.2, 1.1 and 1.6 folds respectively, while both tyrosine transaminase and aspartate transaminase were induced after water-stress.

  A total of four other enzymes identified in the valine, leucine and isoleucine biosynthesis pathway were also up-regulated ranging from 1.5 – 4.9 folds (*Table 4*). However, in the lysine biosynthesis pathway, dihydrodipicolinate synthase was down-regulated 1.3 folds while acetylornithine transaminase was up-regulated 1.0 fold. The increase in the synthesis of amino acids could contribute to human health while the enzymes induced could play a part in protecting the plant from damage.

- **Other biosynthesis pathways**
  1. **Biosynthesis of vitamins**
     Tocopherol cyclase (Vitamin E), gamma tocopherol methyltransferase and ascorbate peroxidase (APX) were up-regulated 1.9, 1.5 and 1.1 folds respectively (*Table 4*). Some of the important enzymes involved in the production of health-benefiting compounds include vitamin C (ascorbic acid), vitamin E (tocopherols) and plant sterols. In addition to their primary role in plants, these phytochemicals are considered as antioxidant nutrients. Vitamins C and A, which are made in the body from beta-carotene, are both needed for the proper functioning of a healthy immune system. Antioxidant properties of both α- and γ-tocopherols (vitamin E) have been shown to play important roles in exerting positive effects on human health. Previous studies on animal models and human colon cancer cell lines have shown that vitamin E may help in preventing colon cancer by reducing the mutagen as a result of free radical oxidation activities (Stone et al. 2004).

  2. **Streptomycin biosynthesis**
     Hexokinase associated with streptomycin biosynthesis was up-regulated 1.2 folds while inositol-3-phosphate synthase was down-regulated 1.6 folds.

  3. **Alkaloid biosynthesis**
     Carboxylesterase (CXE) involved in tropane piperidine and pyridine alkaloid biosynthesis and catechol O-methyltransferase involved in isoquinoline alkaloid biosynthesis were both down-regulated 1.3 and 4.3 folds respectively.

  4. **Enzymes involved in antioxidant defense mechanisms**
     We also identified enzymes involved in enzymatic antioxidant defense mechanisms such as SOD, GR, GST,

Table 4. Differential expression of putative genes identified from the amino acids, streptomycin and other biosynthesis pathways in *Phyllanthus urinaria* under water-stress

Biosynthesis pathways and putative genes identified (including isoforms)	Frequency of 21-bp sequence tags		Fold increase/ decrease	Biosynthesis pathways and putative genes identified (including isoforms)	Frequency of 21-bp sequence tags		Fold increase/ decrease
	Control	Water-stress			Control	Water-stress	
Phenylalanine, tyrosine and tryptophan biosynthesis				**Streptomycin biosynthesis**			
Tyrosine transaminase [2.6.1.5]	–	3	–	Hexokinase [2.7.1.1]	715	849	1.2
Aspartate transaminase [2.6.1.1]	–	14	–	Inositol-3-phosphate synthase [5.5.1.4]	1,229	779	1.6
Tryptophan synthase [4.2.1.20]	244	274	1.1				
Chorismate synthase [4.2.3.5]	124	144	1.2	**Enzymes involved in antioxidant activity**			
Catalase (CAT) [1.11.1.6]	9,259	15,055	1.6	Superoxide dismutase (SOD) [–]	128	356	2.8
				Glutathione reductase (GR) [–]	372	915	2.5
				Glutathione-S-transferase (GST) [–]	229	515	2.3
Valine, leucine and isoleucine biosynthesis				Monodehydroascorbate reductase (MDHAR) [1.6.5.4]	1,430	1,669	1.2
2-isopropylmalate synthase [2.3.3.13]	172	265	1.5				
3-isopropylmalate dehydrogenase 8 39 4.9 [1.1.1.85]	8	39	4.9	Dehydroascorbate reductase (DHAR) [–]	399	828	2.1
Dihydroxy-acid dehydratase [4.2.1.9]	8	34	4.3				
Ketol-acid reductoisomerase [1.1.1.86]	33	52	1.6	**Alkaloid biosynthesis**			
				Carboxylesterase(CXE) [3.1.1.1]	790	602	1.3
Lysine biosynthesis				Catechol O-methyltransferase [–]	14,122	3,262	4.3
Dihydrodipicolinate synthase [4.2.1.52]	334	257	1.3				
Acetylornithine transaminase [–]	57	59	1.0	**Biosynthesis of Vitamins**			
				Ascorbate peroxidase (APX) [1.11.1.11]	1,182	1,330	1.1
				Tocopherol cyclase (Vitamin E) [–]	103	195	1.9
				Gamma tocopherolmethyltransferase [–]	151	233	1.5

*Enzyme commission numbers in [ ] in secondary metabolite biosynthesis pathways in KEGG database

MDHAR and DHAR which were up-regulated 1.2 – 2.8 folds (*Table 4*).

Functional annotation using GO analysis

The total number of sequence tags annotated by Blast2GO analysis in the water-stressed population (48,991) was 1.8 folds more than in the control population (26,838) (*Table 5*). The remaining 116,481 and 236,135 sequence tags in the control and water-stressed populations respectively, either had no match to the sequences in the SwissProt database (E-value $\leq 10^{-6}$), were unclassified (hit to proteins without a GO identifier) or mapped, but not annotated.

Table 6 shows the GO functional classification of the sequence tags in the 3 main categories, BP, MF and CC. Many of the sequences were represented in more than one category.

In BP category, majority of the sequences were represented in the metabolic and cellular processes putative functions. The number of 21-bp sequence tags was 1.5 – 2.5 folds more in all the biological processes in the water-stressed population compared to the control except for the biological adhesion putative function which appeared only after water-stress treatment. This showed that the water-stress conditions caused alterations in the metabolic and cellular processes, thus increasing the number of genes involved to tolerate and protect the plant from damage due to the limiting conditions.

In the MF category, the most highly represented groups were binding and catalytic activity putative functions. The number of 21-bp sequence tags was 1.5 – 2.4 folds more in all the molecular functions in the water-stressed population compared to the control. This showed that growth and development of the plants were altered under water-stress conditions and that these activities became more important for the cell to survive major metabolic changes (Botton et al. 2008). Interestingly, there was a 1.7 folds increase in antioxidant activity putative function in the MF category in the water-stressed population compared to the control. The enzymes in this reactive oxygen scavengers (ROS) group included class III peroxidase (*Table* 2), SOD, GST and APX (*Table 4*). Polyphenoloxidases (PPO) such as plant POD and respiratory burst oxidase genes in the cell wall have been reported to be involved in the oxidation of O-diphenols and synthesise lignins which are crucial for xylem formation, water impermeability and protection against chemical and biological degradation.

Table 5. Blast2GO analysis of sequence tags that matched to PlantTA database in control and water-stressed libraries after blast to SwissProt database (E-value $\leq 10^{-6}$)

	No. of sequence tags	
	Control	Water-stress
B2G annotated	26,838	48,991
Unclassified	60,912	119,707
Mapped but not annotated	43,427	88,364
With no match	12,142	28,064
Total	143,320	285,127

In the CC category, majority of the sequences mapped to the cell and organelle putative functions. Localisation of the tags to the cell and organelle components aids in characterisation and mapping of genes. The number of 21-bp sequence tags was 1.8 – 2.3 folds more in all the putative functions in the water-stressed population compared to the control except for the synapse group which was induced only after water-stress. Liu et al. (2005) reported that most of the genes in this category are usually involved in energy and secondary metabolite production. Localisation of the tags to the cell and organelle components aids in characterisation and mapping of genes.

Conclusion

Our data has demonstrated that water-stress triggered transcriptional changes in the expression levels of several useful genes in the secondary metabolite biosynthesis

Table 6. GO functional classification of control and water-stressed *Phyllanthus urinaria* sequence tags using Blast2GO at level 2 (E-value ≤1 x 10^{-6})

Category	Putative functions/GO terms	No. of 21-bp sequence tags		
		Control	Water-stressed	Fold-increase
Biological process (BP)	Metabolic process	13,693	23,717	1.7x
	Cellular process	13,242	22,969	1.7x
	Localization	2,261	4,352	1.9x
	Biological regulation	1,894	3,610	1.9x
	Response to stimulus	1,633	3,275	2.0x
	Developmental process	494	972	2.0x
	Multicellular organismal process	430	863	2.0x
	Reproduction	185	394	2.1x
	Multi-organism process	175	352	2.0x
	Growth	60	148	2.5x
	Immune system process	60	148	2.5x
	Locomotion	33	64	1.9x
	Viral reproduction	16	37	2.3x
	Pigmentation	15	23	1.5x
	Biological adhesion	0	18	–
Molecular function (MF)	Binding	14,563	26,122	1.8x
	Catalytic activity	12,352	22,324	1.8x
	Structural molecule activity	1,353	2,335	1.7x
	Transporter activity	961	1,792	1.9x
	Electron carrier activity	696	1,191	1.7x
	Translation regulator activity	528	810	1.5x
	Transcription regulator activity	324	607	1.9x
	Antioxidant activity	284	487	1.7x
	Enzyme regulator activity	194	456	2.4x
	Molecular transducer activity	148	303	2.1x
	Nutrient reservoir activity	112	224	2.0x
Celullar component (CC)	Cell	15,940	28,503	1.8x
	Organelle	10,358	18,500	1.8x
	Macromolecular complex	3,545	6,064	1.7x
	Envelope	588	1,115	1.9x
	Membrane-enclosed lumen	562	1,145	2.0x
	Extracellular region	338	763	2.3x
	Virion	9	16	1.8x
	Synapse	0	13	–

pathways in *P. urinaria* that not only protects the plant but also contributes to human health. The DGE technology approach together with bioinformatics tools have allowed us to acquire these changes on a large-scale at a genome-wide level and to identify genes that showed response to drought, even for low abundance transcripts. However, the limitations of the DGE technology include only sampling genes with *Nla*III sites within their open reading frames, an under representation of genes with long 3' untranslated regions (UTRs) and the inability to align many tags uniquely to the transcriptome. While DGE allows transcription profiling of a large proportion of the genes in the genome despite the unavailability of a genomic sequence for a non-model plant species like *P. urinaria*, a more comprehensive analysis of the transcriptome could be obtained by the RNA sequencing (RNAseq) approach. Overall, this study may provide a foundation for future studies on over expression of specific genes for production of useful secondary metabolites associated with health benefits. Further studies have been carried out using UV-B stress treatment to enhance these useful secondary metabolites biosynthesis in this plant and compare its effectiveness to water-stress.

Acknowledgment

The authors would like to thank Mr Chandradevan Machap for providing us with the water-stressed and control *P. urinaria* plants. The authors are also grateful to Mr Lee Weng Wah, Ms Sufiah Sebaweh and Ms Aida Al-Quswa for assisting us in the bioinformatics analysis of the genomics data. This study was supported by the Ministry of Science, Technology and Innovation of Malaysia (MOSTI) under the Grant No. 02-03-08-SF0171.

References

Ahmad, P., Jaleel, C.A., Azooz, M.M. and Nabi, G. (2009). Generation of ROS and non enzymatic antioxidants during abiotic stress in plants. *Botany Research International* 2(1): 11 – 20

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M. and Sherlock, G. (2000). Gene ontology: tool for the unification of biology. The gene ontology consortium. *Nat Genet* 25: 25 – 29

Botton, A., Galla, G., Conesa, A., Bachem, C., Ramina, A. and Barcaccia, G. (2008). Large-scale gene ontology analysis of plant transcriptome-derived sequences retrieved by AFLP technology. *BMC Genomics* 9: 347

Calixto, J.B., Santos, A.R., Filho, V.C. and Yunes, R.A. (1998). A review of the plants of the genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. *Med Res Rev* 18: 225 – 258

Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. *Photochem. Photobiol*. 70: 1 – 9

Chang, C.C., Lien, Y.C., Liu, K.C. and Lee, S.S. (2003). Lignans from *Phyllanthus urinaria*. *Pytochemistry* 63: 825 – 833

Chang, S.J., Puryear, J. and Cairney, J. (1993). A simple and efficient method for isolating RNA from pine trees. *Plant Molecular Biology* 11(2): 113 – 116

Childs, K.L., Hamilton, J., Zhu, W., Ly, E., Cheung, F., Wu, H., Rabinowicz, P.D., Town, C.D., Buell, C.R. and Chan, A.P. (2007). The TIGR plant transcript assemblies database. *Nucleic Acids Res*. 35: D846 – D851 (Database issue doi:10.1093/nar/gkl785)

Conesa, A., Gotz, S., Garcia-Gomez, J.M., Terol, J., Talon, M. and Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21(21): 3674 – 3676

Dixon, R.A. and Paiva, N.L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell*. 7: 1085 – 1097

Gene Ontology Consortium (2006). The gene ontology (GO) project. *Nucleic Acids Res* 34: D322 – D326

Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48: 909 – 930

Gotz, S., Garcia-Gomez, J.M., Terol, J., Williams, T.D., Nagaraj, S.H., Nueda, M.J., Robles, M., Talon, M., Dopazo, J. and Conesa, A. (2008). High throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Res* 36: 3420 – 3435

Huang, S.T., Yang, R.C., Lee, P.N., Yang, S.H., Liao, S.K. Chen, T.Y. and Pang, J.H.S. (2006). Anti-tumor and anti-angiogenic effects of *Phyllanthus urinaria* in mice bearing Lewis lung carcinoma. *International Immunopharmacology* 6: 870 – 879

Indrajith, A. and Ravindran, K.C. (2009). Antioxidant potential of Indian medicinal plant *Phyllanthusamarus* L. under supplementary UV-B radiation. *Recent Research in Science and Technology* 1(1): 34 – 39

Laila, M.A. (2006). The nature-versus-nurture debate on bioactive phytochemicals: the genome versus terroir. *J. Sci. Food Agri.* 86(15): 2510 – 2515

Le, D.T., Nishiyama, R., Watanabe, Y., Tanaka, M. and Seki, M. (2012). Differential gene expression in soybean leaf tissues at late developmental stages under drought stress revealed by genome-wide transcriptome analysis. *PLoS ONE* 7(11): e49522. doi:10.1371/journal.pone.0049522

Liang, B., Huang, X., Zhang, G. and Zhou, Q. (2006). Effect of Lanthanum on plant under supplementary ultraviolet-B radiation: Effect of Lanthanum on flavonoid contents on soybean seedling exposed to supplementary ultraviolet-B radiation. *J. Rare Earth* 24: 613 – 616

Liu, K.C., Lin, M.T., Lee, S.S., Chion, J.F., Ren, S. and Lien, E.J. (1999). Antiviral tannins from two *Phyllanthus* species. *Planta Medica* 65: 43 – 46

Liu, Y., Wang, H., He-Chun, Y.E. and Guo-Feng, L.I. (2005). Advances in the plant isoprenoid biosynthesis pathway and its metabolic engineering. *J. Integr Plant Biol* 47: 769 – 782

Matsui, A., Ishida, J., Morosawa, T., Mochizuki, Y. and Kaminuma, E. (2008). *Arabidopsis* transcriptome analysis under drought, cold, high-salinity and ABA treatment conditions using a tiling array. *Plant Cell Physiol* 49: 1135 – 1149

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S. and Devasagayam, T.P.A. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *J. Clin. Biochem. Nutr.* 40: 163 – 173

Penuelas, J. and Munne-Bosch, S. (2005). Isoprenoids: an evolutionary pool for photoprotection. *Trends Plant Sci* 10: 166 – 169

Philippe, N., Elias, B.S., Anthony, B., Alban, M., Florence, R., Qiang, B., John, De V., Eric, R. and Therese, C. (2013). Combining DGE and RNA-sequencing data to identify new polyA$^+$non-coding transcripts in the human genome. *Nucleic Acids Research* 42(5): 2820 – 2832

Polovka, M., Brezova, V. and Stasko, A. (2003). Antioxidant properties of tea investigated by EPR spectroscopy. *Biophys. Chem.* 106: 39 – 56

Reddy, P.C.O., Sairanganayakulu, G., Thippeswamy, M., Sudhakar Reddy, P., Reddy, M.K. and Sudhakar, C. (2008). Identification of stress-induced genes from the drought tolerant semi-arid legume crop horsegram (*Macrotyloma uniflorum* Lam. Verdc.) through analysis of subtracted expressed sequence tags. *Plant Sci.* doi:10.1016/j.plantsci.2008.05.012

Sakihama, Y., Cohen, M.F., Grace, S.C. and Yamasaki, H. (2002). Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. *Toxicology* 177: 67 – 80

Satyan, K.S., Prakash, A., Singh, R.P. and Srivastava, R.S. (1995). Phthalic acid bis-ester and other phytoconstituents of *Phyllanthus urinaria*. *Planta Medica* 61: 293 – 294

Sharma, S.S. and Dietz, K.J. (2006).The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *Journal of Experimental Botany* 57(4): 711 – 726

Shendure, J. and Ji, H. (2008). Next-generation DNA sequencing. *Nat. Biotechnol* 26(10): 1135 – 1145

Stone, W.L., Krishnan, K. and Campbell, S.E. (2004). Tocopherols and the treatment of colon cancer. *Ann NY Acad Sci*. 1031: 223 – 233

Veitch, N.J., Johnson, P.C.D., Trivedi, U., Terry, S., Wildridge, D. and MacLeod, A. (2010). Digital gene expression analysis of two life cycle stages of the human-infective parasite, *Trypanosoma brucei gambiense* reveals differentially expressed clusters of co-regulated genes. *BMC Genomics* 11: 124

Wang, C.Y. and Lee, S.S. (2005). Analysis and identification of lignans in *Phyllanthus urinaria* by HPLC-SPE-NMR.*Phytochemical Analysis* 16: 120 – 126

Yadav, R.K., Sangwan, R.S., Sabir, F., Srivastava, A.K. and Sangwan, N.S. (2013). Effect

of prolonged water stress on specialized secondary metabolites, peltate glandular trichomes, and pathway gene expression in *Artemisia annua* L. *Plant Physio Biochem*. 74C: 70 – 83 doi: 10.1016/j.plaphy.2013.10.023

Yamaguchi, S.K. and Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu Rev Plant Biol* 57: 781 – 803

Yamasaki, H., Heshiki, R. and Ikehara, N.(1995). Leaf-goldening induced by high light in *Ficusmicrocarpa* L. f., a tropical fig. *J. Plant Res*. 108: 171 – 180

Yang, C.M., Cheng, H.Y., Lin, T.C., Chiang, L.C. and Lin, C.C. (2005). Acetone, ethanol and methanol extracts of *Phyllanthus urinaria* inhibits HSV-2 infection *in-vitro*. *Antiviral Research* 67: 24 – 30

—— (2007). The *in vitro* activity of geraniin and 1,3,4,6-tetra-*O*-galloyl-β-D-glucose isolated from *Phyllanthus urinaria* against herpes simplex virus type 1 and type 2 infection. *Journal of Ethnopharmacology* 110: 555 – 558

Zhou, J., Wang, X., Jiao, Y., Qin, Y. and Liu, X. (2007). Global genome expression analysis of rice in response to drought and high-salinity stresses in shoot, flag leaf and panicle. *Plant Mol Biol* 63: 591 – 608

Abstrak

Kajian ini telah dijalankan untuk mengkaji pengekspresan gen-gen yang terlibat dalam tapak jalan penghasilan metabolit sekunder dalam tumbuhan dukung anak (*Phyllanthus urinaria*) dalam keadaan kekurangan air di dalam rumah jaring. Tumbuhan ini telah dikenal untuk menghasilkan bahan fitokimia yang berguna pada kesihatan manusia. Analisis data mentah dengan menggunakan teknologi ekspressi gen digital (DGE) menunjukkan nombor 'tag' jujukan dalam pokok di bawah penekanan air adalah 1.9 kali ganda berbanding dengan pokok kawalan. Analisis "Blast" menunjukkan lebih daripada 90% jujukan ini telah dikenal pasti dengan jujukan dalam pangkalan data plantTA. Ekspresi beberapa gen yang terlibat dalam tapak jalan biosintesis utama iaitu isoprenoid, shikimate, asid lemak, steroid dan asid amino didapati meningkat atau menurun dalam pokok yang didedah kepada keadaan kekurangan air berbanding dengan pokok kawalan. Didapati juga beberapa gen yang teraruh oleh penekanan air tetapi dengan frekuensi yang rendah manakala ada beberapa yang hilang langsung atau tidak menunjukkan perubahan dalam ekspresinya. Analisis 'Gene Ontology (GO)' pada paras 2 menunjukkan jujukan yang lebih dan signifikan (E value $\leq 10^{-6}$) dalam pokok yang mengalami penekanan air berbanding dengan pokok kawalan di semua kategori 'biological process, molecular function' dan 'cellular component'. Kajian ini menunjukkan kesan kebaikkan kekurangan air dalam penghasilan beberapa metabolit sekunder berguna dalam tumbuhan dukung anak.