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Research Paper

Comparative transcriptome of purple and white bracts of *Bougainvillea glabra* 'Mrs. Eva'

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Abstract

Bract colour is the most important ornamental trait of *Bougainvillea*. To explore the molecular mechanism of its colour formation, the present study sequenced the transcriptome of purple bracts and its white mutation. A total of 3762 up and 3306 down-regulated genes were detected in the purple bracts compared with the control. The differentially expressed genes (DEGs) mainly participate in flavone and flavonol biosynthesis, vitamin B6 metabolism and nitrogen metabolism. Except vitamin B6 metabolism, the DEGs were mainly up-regulated in these pathways and GO enrichment analysis confirmed these findings. Ninety three (93) differentially expressing transcription factors were found in the comparison, which mainly contributed to MYB, MYB-related and C3H families.

Keywords: Bougainvillea, Transcriptome, Betalain, Differentially expressed genes (DEGs)

Introduction:

Bougainvillea is a type of Caryophyllales ornamental plant in the tropical and sub-tropical parts of the world. The three bracts locating around the flower are rich in betalains which exhibiting gorgeous and diversiform colours (Roy *et al*., 2015). The purple bracts of *Bougainvillea* only contain betacyanins, the yellow and orange bracts contain a principal component of betaxanthins and a small amount of betacyanins, and the red bracts contain equal proportion of these two kinds of betalains (Kugler *et al*., 2007). While the bracts without colourful betalains commonly show light green to white colour.

The 'Mrs. Eva' series is a class of widely cultivated *Bougainvillea* variety, besides the wild type 'Mrs. Eva' with purple bracts and full green leaves. It also contains many other derived varieties with change morphological characters, including white bract type, mottled bract type, variegated leaf type and so on, which all contributed to mutation germplasm (RHS, 2013). In order to detect the transcriptional difference between betacyanin-containing and non-betacyanin bracts of *Bougainvillea*, the present study selected and performed a standard transcriptome sequencing besides comparative analysis on the wild *Bougainvillea* 'Mrs. Eva' and its white bract mutation.

Material and Methods:

Transcriptome Sequencing – A six years old *Bougainvillea* 'Mrs. Eva' plant containing both wild branches (purple bracts) and natural mutated branches (white bracts) (Figure - 1) were selected and sampled. Six bracts from the flowering inflorescences were collected randomly as a biological repetition and each bract type was prepared three repetitions. RNAs were extracted, an oligo (dT) method was used to enrich mRNAs and the library was sequenced on a BGISEQ-500 platform (BGI, Shenzhen, China).

Bioinformatics Analysis - The low quality reads and reads with adaptors and unknown bases were filtered using SOAPnuke (https://github.com/BGI-flexlab/SOAPnuke). The clean reads were mapped to a reference mRNA database of 'Mrs. Eva' sequenced from a PacBio platform (in house, not shown) using Bowtie2 (https://sourceforge.net/projects/bowtie-bio/files/bowtie2/). The gene expression level was calculated using RSEM (https://deweylab.github.io/RSEM/), which is a package to estimating gene and isoform expression levels. The DEGs were detected using DEGseq (https://www.bioconductor.org/packages/release/bioc/html/DEGseq.html) based on the poisson distribution.

The DEGs were annotated in KEGG (https://

www.genome.jp/kegg) and GO (https://geneontology.org), and were classified according to their official classification catalogues. The KEGG and GO functional enrichment were performed using phyper, which is a function of R language. The P-value was calculated in hypergeometric test as following:

$$P = 1 - \sum_{i=0}^{m-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

The false discovery rate (FDR) for each P-value were calculated and the terms which FDR not larger than 0.01 were defined as significant enriching. The ORFs were identified using getorf (http://emboss.sourceforge.net/apps/cvs/emboss/apps/getorf.html) from each DEG and aligned to transcriptional factor (TF) domains in PlntfDB (http://plntfdb.bio.uni-potsdam.de) using hmmsearch (https://www.ebi.ac.uk/Tools/hmmer/search/hmmsearch).

Results and Discussion:

The DEGs between Purple and White Bracts - Six samples were sequenced and averagely generated about 65.76 M reads per sample. The proportion of clean reads with Q30 reached to 90% above (Table 1). Based on the gene expression level from SOAPnuke, the differentially expressed genes (DEGs) between purple and white bracts were identified. In contrast to the non-betacyanin bract, 3762 genes and 3306 genes were up- and down-regulated in the betacyanin containing bract, respectively (Figure - 2, Table S1). The most up-regulated genes with known function and $\log_2$ratio > 9.7 are *Bou_89304*, *Bou_53094*, *Bou_38889* and *Bou_79967*, while the most down-regulated genes with known function and $\log_2$ratio < -9.7 are *Bou_10998*, *Bou_4239*, *Bou_8379* and *Bou_12239*. The *Bou_89304* and *Bou_53094* genes encode membrane structure proteins, the former one encodes curvature thylakoid 1A which located in chloroplast, and the later one encodes endoplasmin which plays critical roles in folding proteins in the secretory pathway (Klein *et al.*, 2006). The *Bou_38889* gene encodes lipoxygenase which catalyzes the deoxygenation of polyunsaturated fatty acids in lipids (Porta and Rochasosa, 2002), and the *Bou_79967* gene encodes elicitor inducible protein. The *Bou_10998* gene encodes NAD(P)H-quinone oxidoreductase subunit H which participates in the energy metabolism. The *Bou_4239* gene encodes brefeldin A-inhibited guanine nucleotide-exchange protein which is essential for regulating vesicular traffic among intracellular organelles (Citterio *et al.*, 2006). The *Bou_8379* and *Bou_12239* genes all encode E3 ubiquitin protein ligase upl2 (uniprot: Q8H0T4) that directly catalyzes the transfer of ubiquitin from E2 to the protein substrate. Five betalain-/anthocyanidin-biosynthesis genes were detected as DEGs, including dihydroflavonol 4-reductase coding genes (DFR, *Bou_72733* and *Bou_23972*), and cytochrome P 450 76AD3 coding genes (CYP76AD3, *Bou_83735*, *Bou_71439* and *Bou_31576*). DFR locates in the downstream steps of anthocyanin biosynthesis pathway, which is not expressed in most tissues and organs, except seeds of the betalain-producing taxa such as *Spinacia oleracea* and *Phytolacca americana* (Shimada *et al.*, 2005). Xu *et al.* (2016) found its higher expression in the red bracts of *Bougainvillea* compared with the white bracts, and an unstable relative expression in the purple bract compared with the white bracts in different development stages. The present study for the bracts at the flowering stage found its down-regulation expression. CYP76AD3 gene consists of one intron and two exons in *Mirabilis jalapa*, which affects a gene encoding an enzyme for betalain synthesis that results in a variegated flower phenotype (Suzuki *et al.*, 2014). The present study found its up-regulated expression in the purple bracts.

Fig. 1. Purple and white bracts of *Bougainvillea glabra* 'Mrs. Eva'

Table 1. Clean reads quality metrics of bract transcriptome of *Bougainvillea glabra*

Sample	Total Clean Reads (Mb)	Total Clean Bases (Gb)	Clean Reads Q20 (%)	Clean Reads Q30 (%)	Clean Reads Ratio (%)
Purple_1	69.73	6.52	97.6	90.78	93.56
Purple_2	69.73	6.55	97.58	90.84	93.92
Purple_3	69.73	6.55	97.59	90.65	93.97
White_1	72.22	6.71	97.46	90.45	92.9
White_2	69.73	6.56	97.72	91.22	94.06
White_3	69.73	6.56	97.62	90.82	94.15

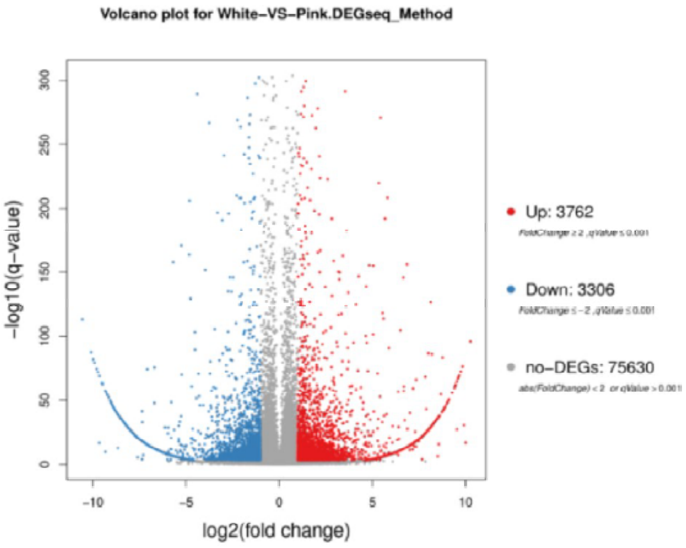

Fig. 2. Volcano plot of DEGs

X–axis represents log2 transformed fold change. Y–axis represents -log10 transformed significance. Red points represent up-regulated DEGs. Blue points represent down-regulated DEGs. Gray points represent non-DEGs.

Pathway Analysis of Differential Expressed Genes - Most of DEGs participate in the metabolic pathways, the rich factor of pathways including flavone and flavonol biosynthesis, non-homologous end-joing, vitamin B6 metabolism and nitrogen metabolism reached to 0.15 above (Figure 3), which indicates a widely transcriptional change in these pathways. Except vitamin B6 metabolism, the DEGs are mainly up-regulated in these pathways (Figure 4). It should note that the betalain metabolism pathway isn't clear enough till now that isn't collated by the KEGG. The betalain and anthocyanin productions are mutually exclusive in the plant kingdom (Brockington *et al*., 2011; Harris *et al*., 2012), but it's also found the different expression of flavone biosynthesis pathways and regulation genes exhibited above in *Bougainvillea* bracts, which also found in the study of Xu *et al*. (2016) that compared the transcriptome of red *B. glabra* Choisy. 'Sanderiana', white *B. glabra* 'Alba', orange *B. buttiana* 'Salmoea' and purple *B. glabra* 'Formosa'. The vitamin B6 has been proved as a reactive oxygen species scavenger, and has potential ability to increase resistance to both biotic and abiotic stresses, while the betalain has similar function (Mooney and Hellmann, 2010; Jain and Gould, 2015). The up-regulation of vitamin B6 pathway indicate a function substitution of vitamin B6 to betacyanins in the white bracts.

The numbers of up-regulated DEGs are also commonly more than the down-regulated DEGs in the GO terms, except rhythmic process, extracellular region, antioxidant activity, nutrient reservoir activity and transcription factor activity (Figure 5). It has been proved that the transcription factor (TF) can regulate the biosynthesis of the plant pigments. Hatlestad *et al*. (2015) found a MYB-type transcription factor in red beet can act as positive regulator of betalain biosynthesis. The present study identified 93 differential expressed TFs, which mainly contribute to C3H, MYB and MYB-related types (Table S2). Compared with the white bracts, the TFs in these types are mainly down-regulated in the purple bracts (Figure 6). Most of the identified TFs maintain a low expression level, only seven TFs are with FPKM larger than 400, including *Bou_43856*, *Bou_51116*, *Bou_28237*, *Bou_27025*, *Bou_61726*, *Bou_11000* and *Bou_71140*. The TF type and relative expression level of these genes don't show significant rules.

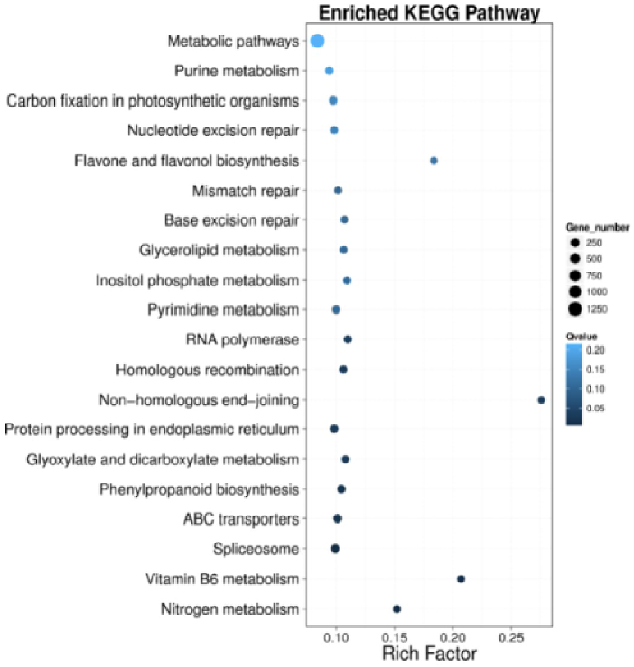

Fig. 3. Pathway functional enrichment of DEGs.

X–axis represents enrichment factor. Y–axis represents pathway name. The color indicates the q-value (high: white, low: blue), the lower q-value indicates the more significant enrichment. Point size indicates DEG number (The bigger dots refer to larger amount). Rich Factor refers to the value of enrichment factor, which is the quotient of foreground value (the number of DEGs) and background value (total Gene amount). The larger the value, the more significant enrichment.

Table 1. The DEGs between Purple and White Bracts

Gene ID	**Length**	**White-Expression**	**Pink-Expression**	**log2Ratio(Pink/White)**	**log2Ratio(Pink/White)**	**q-value(Storey et al. 2003)**	**Up-Down-Regulation(Pink/White)**
Bou_89304	458	0	102.63	10.001592	10.001592	1.68E-17	Up
Bou_10998	5153	503.51	0	-9.977521	9.977521	7.93E-83	Down
Bou_53094	1026	0	184.32	9.9203531	9.9203531	4.85E-31	Up
Bou_4239	5657	482.73	0	-9.916717	9.9167171	3.90E-80	Down
Bou_38889	1413	0	460.14	9.8442847	9.8442847	4.02E-77	Up
Bou_79967	571	0	458.32	9.8385671	9.8385671	7.00E-77	Up
Bou_8379	5305	444.67	0	-9.798236	9.7982356	4.00E-75	Down
Bou_12239	5095	424.98	0	-9.732896	9.7328955	1.70E-72	Down
Bou_72733	681	51.38	0	-9.328636	9.3286356	4.28E-10	Down
Bou_83735	520	0	35.65	6.1541858	6.1541858	2.99E-09	Up
Bou_71439	703	30.92	178.69	2.5292023	2.5292023	1.64E-25	Up
Bou_31576	1716	4325.17	19489.99	2.1702602	2.1702602	0	Up
Bou_23972	2341	245.22	105.35	-1.220531	1.2205307	2.51E-13	Down
Bou_1598	6119	755.71	0	-10.56333	10.563333	1.70E-113	Down
Bou_53863	1011	0	613.76	10.259886	10.259886	1.39E-96	Up
Bou_47367	1161	543.62	0	-10.0881	10.088099	6.11E-88	Down
Bou_12677	5075	0	428.98	9.7431222	9.7431222	5.53E-73	Up
Bou_98630	336	96.92	0	-9.659261	9.6592606	2.20E-17	Down
Bou_48537	1131	0	404.35	9.6578164	9.6578164	1.19E-69	Up
Bou_75392	639	400.81	0	-9.648419	9.6484191	3.24E-69	Down
Bou_57798	938	0	395.09	9.6243931	9.6243931	2.19E-68	Up
Bou_56797	957	0	387.95	9.5980825	9.5980825	2.09E-67	Up
Bou_78903	586	0	156.2	9.537145	9.537145	7.31E-28	Up
Bou_60421	891	0	371.82	9.5368162	9.5368162	3.58E-65	Up
Bou_64230	824	363.55	0	-9.507654	9.5076544	4.64E-64	Down
Bou_10719	5168	362.99	0	-9.50543	9.5054304	5.56E-64	Down
Bou_5523	5521	357.42	0	-9.483121	9.4831209	3.37E-63	Down
Bou_14349	5003	0	354.3	9.4671833	9.4671833	1.02E-62	Up
Bou_5730	5505	0	353.76	9.4649827	9.4649827	1.22E-62	Up
Bou_11695	5119	0	351.74	9.4567212	9.4567212	2.35E-62	Up
Bou_51806	1054	0	349.11	9.4458935	9.4458935	5.51E-62	Up
Bou_68794	747	0	339.3	9.4047732	9.4047732	1.35E-60	Up
Bou_9558	5229	322.27	0	-9.33377	9.3337705	3.38E-58	Down
Bou_6420	5448	317.51	0	-9.312303	9.3123026	1.64E-57	Down
Bou_2705	5877	313.94	0	-9.295989	9.2959894	5.41E-57	Down
Bou_42619	1294	0	310.75	9.2779662	9.2779662	1.72E-56	Up
Bou_34391	1581	299.98	0	-9.230367	9.2303669	5.95E-55	Down
Bou_14509	4997	298.7	0	-9.224198	9.2241978	9.16E-55	Down

Gene ID	**Length**	**White-Expression**	**Pink-Expression**	**log2Ratio(Pink/White)**	**log2Ratio(Pink/White)**	**q-value(Storey et al. 2003)**	**Up-Down-Regulation(Pink/White)**
Bou_66033	793	285.76	0	-9.160305	9.1603046	7.43E-53	Down
Bou_65362	805	0	275.53	9.1044212	9.1044212	2.71E-51	Up
Bou_86281	493	0	275.34	9.103426	9.103426	2.89E-51	Up
Bou_5874	5492	270.51	0	-9.081182	9.0811825	1.42E-50	Down
Bou_55878	974	0	264.43	9.0450977	9.0450977	1.27E-49	Up
Bou_20075	4657	262.84	0	-9.039685	9.0396854	2.04E-49	Down
Bou_1831	6060	262.51	0	-9.037873	9.0378729	2.29E-49	Down
Bou_20361	4574	261.3	0	-9.031208	9.0312077	3.49E-49	Down

p-value	**Cellular Component**	**Molecular Function**	Biological Process	Kegg Orthology	Nr Description
1.72 E-18	GO:0009535//chloroplast thylakoid membrane;GO:0010287//plastoglobule;GO:0016021//integral component of membrane;GO:0009941//chloroplast envelope	NA	NA	NA	gi|702327324|ref|XP_010053778.1|//PREDICTED: protein CURVATURE THYLAKOID 1A, chloroplastic [Eucalyptus grandis]
1.64 E-84	GO:0016021//integral component of membrane;GO:0009579//thylakoid	GO:0046872//metal ion binding;GO:0051539//4 iron, 4 sulfur cluster binding;GO:0048038//quinone binding;GO:0051287//NAD binding;GO:0008137//NADH dehydrogenase (ubiquinone) activity	GO:0055114//oxidation-reduction process	K05579//NAD(P)H-quinone oxidoreductase subunit H [EC:1.6.5.3]	gi|357467749|ref|XP_003604159.1|//NAD(P)H-quinone oxidoreductase subunit H [Medicago truncatula]
2.79 E-32	NA	NA	NA	K09487//heat shock protein 90kDa beta	gi|698490000|ref|XP_009791518.1|//PREDICTED: endoplasmin homolog [Nicotiana sylvestris]
8.32 E-82	NA	GO:0005086//ARF guanyl-nucleotide exchange factor activity	GO:0032012//regulation of ARF protein signal transduction;GO:0043547//positive regulation of GTPase activity	K18442//brefeldin A-inhibited guanine nucleotide-exchange protein	gi|225428344|ref|XP_002280001.1|//PREDICTED: brefeldin A-inhibited guanine nucleotide-exchange protein 2-like [Vitis vinifera]
8.88 E-79	NA	GO:0016702//oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen;GO:0046872//metal ion binding	GO:0006633//fatty acid biosynthetic process;GO:0055114//oxidation-reduction process;GO:0031408//oxylipin biosynthetic process	K00454//lipoxygenase [EC:1.13.11.12]	gi|326418056|gb|ADZ73653.1|//putative 13-lipoxygenase [Capsicum annuum]
1.55 E-78	NA	NA	NA	NA	gi|40287496|gb|AAR83862.1|//elicitor-inducible protein EIG-J7 [Capsicum annuum]
9.07 E-77	NA	GO:0004842//ubiquitin-protein transferase activity;GO:0016874//ligase activity	GO:0016567//protein ubiquitination	K10592//E3 ubiquitin-protein ligase HUWE1 [EC:2.3.2.26]	gi|590605394|ref|XP_007020478.1|//E3 ubiquitin protein ligase upl2, putative isoform 3 [Theobroma cacao]
4.05 E-74	NA	GO:0004842//ubiquitin-protein transferase activity;GO:0016874//ligase activity	GO:0016567//protein ubiquitination	K10592//E3 ubiquitin-protein ligase HUWE1 [EC:2.3.2.26]	gi|359492508|ref|XP_002283711.2|//PREDICTED: E3 ubiquitin-protein ligase UPL2-like [Vitis vinifera]
7.50 E-11	NA	GO:0003854//3-beta-hydroxy-delta5-steroid dehydrogenase activity;GO:0050662//coenzyme binding	GO:0006694//steroid biosynthetic process;GO:0055114//oxidation-reduction process	K09753//cinnamoyl-CoA reductase [EC:1.2.1.44]	gi|703083360|ref|XP_010092193.1|//Bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase [Morus notabilis]
5.73 E-10	GO:0016021//integral component of membrane	GO:0016705//oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen;GO:0020037//heme binding;GO:0005506//iron ion binding;GO:0004497//monooxygenase activity	GO:0055114//oxidation-reduction process	K05280//flavonoid 3'-monooxygenase [EC:1.14.13.21]	gi|356968422|gb|AET43292.1|//CYP76AD3 [Mirabilis jalapa]

p-value	**Cellular Component**	**Molecular Function**	Biological Process	Kegg Orthology	Nr Description
1.16E-26	GO:0016021//integral component of membrane	GO:0016705//oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen;GO:0020037//heme binding;GO:0005506//iron ion binding;GO:0004497//monooxygenase activity	GO:0055114//oxidation-reduction process	K05280//flavonoid 3'-monooxygenase [EC:1.14.13.21]	gi|356968422|gb|AET43292.1|//CYP76AD3 [Mirabilis jalapa]
0	GO:0016021//integral component of membrane	GO:0016705//oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen;GO:0020037//heme binding;GO:0005506//iron ion binding;GO:0004497//monooxygenase activity	GO:0055114//oxidation-reduction process	K00517;K05280//flavonoid 3'-monooxygenase [EC:1.14.13.21]	gi|356968422|gb|AET43292.1|//CYP76AD3 [Mirabilis jalapa]
3.35E-14	NA	GO:0003854//3-beta-hydroxy-delta5-steroid dehydrogenase activity;GO:0050662//coenzyme binding	GO:0006694//steroid biosynthetic process;GO:0055114//oxidation-reduction process	K09753//cinnamoyl-CoA reductase [EC:1.2.1.44]	gi|703083360|ref|XP_010092193.1|//Bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase [Morus notabilis]
2.49E-115	GO:0009570//chloroplast stroma	GO:0016040//glutamate synthase (NADH) activity;GO:0005506//iron ion binding;GO:0010181//FMN binding;GO:0050660//flavin adenine dinucleotide binding;GO:0051536//iron-sulfur cluster binding	GO:0009735//response to cytokinin;GO:0048589//developmental growth;GO:0006537//glutamate biosynthetic process;GO:0055114//oxidation-reduction process;GO:0019676//ammonia assimilation cycle	K00264//glutamate synthase (NADPH/NADH) [EC:1.4.1.13 1.4.1.14]	gi|643706019|gb|KDP22151.1|//hypothetical protein JCGZ_25982 [Jatropha curcas]
2.42E-98	GO:0005840//ribosome	GO:0003735//structural constituent of ribosome	GO:0006412//translation	K02930//large subunit ribosomal protein L4e	gi|703115170|ref|XP_010100833.1|//60S ribosomal protein L4 [Morus notabilis]
1.19E-89	NA	NA	NA	K01051//pectinesterase [EC:3.1.1.11]	gi|661890295|emb|CDP05792.1|//unnamed protein product [Coffea canephora]
1.31E-74	GO:0005643//nuclear pore;GO:0009507//chloroplast	NA	NA	K14310//nuclear pore complex protein Nup205	gi|297738947|emb|CBI28192.3|//unnamed protein product [Vitis vinifera]
2.27E-18	NA	NA	NA	NA	NA
2.93E-71	NA	GO:0008171//O-methyltransferase activity;GO:0030787//inositol 4-methyltransferase activity;GO:0046983//protein dimerization activity	GO:0006020//inositol metabolic process;GO:0032259//methylation	K13066//caffeic acid 3-O-methyltransferase [EC:2.1.1.68]	gi|1170555|sp|P45986.1|IMT1_MESCR//RecName: Full=Inositol 4-methyltransferase [Mesembryanthemum crystallinum]
8.04E-71	GO:0016021//integral component of membrane	GO:0005524//ATP binding;GO:0004222//metalloendopeptidase activity;GO:0008568//microtubule-severing ATPase activity	GO:0006508//proteolysis;GO:0051301//cell division	K03798//cell division protease FtsH [EC:3.4.24.-]	gi|302141673|emb|CBI18876.3|//unnamed protein product [Vitis vinifera]
5.49E-70	NA	NA	NA	K01188//beta-glucosidase [EC:3.2.1.21]	gi|703065069|ref|XP_010087358.1|//Beta-glucosidase 12 [Morus notabilis]
5.33E-69	GO:0005829//cytosol	GO:0016920//pyroglutamyl-peptidase activity	GO:0006508//proteolysis	K01304//pyroglutamyl-peptidase [EC:3.4.19.3]	gi|641819523|gb|KDO39792.1|//hypothetical protein CISIN_1g024368mg [Citrus sinensis]
4.73E-29	NA	NA	NA	K13195//cold-inducible RNA-binding protein;K12897//transformer-2 protein	gi|672173319|ref|XP_008806788.1|//PREDICTED: glycine-rich RNA-binding protein 3, mitochondrial [Phoenix dactylifera]
9.42E-67	GO:0031902//late endosome membrane	GO:0043130//ubiquitin binding;GO:0046872//metal ion binding	GO:0036258//multivesicular body assembly;GO:0070676//intralumenal vesicle formation	K00921//1-phosphatidylinositol-3-phosphate 5-kinase [EC:2.7.1.150]	gi|596141459|ref|XP_007222458.1|//hypothetical protein PRUPE_ppa003456mg [Prunus persica]
1.24E-65	NA	NA	NA	K00454//lipoxygenase [EC:1.13.11.12]	gi|697160377|ref|XP_009588961.1|//PREDICTED: linoleate 13S-lipoxygenase 2-1, chloroplastic-like [Nicotiana tomentosiformis]

p-value	**Cellular Component**	**Molecular Function**	Biological Process	Kegg Orthology	Nr Description
1.48E-65	GO:0030132//clathrin coat of coated pit;GO:0030130//clathrin coat of trans-Golgi network vesicle	GO:0005198//structural molecule activity	GO:0006886//intracellular protein transport;GO:0016192//vesicle-mediated transport	K04646//clathrin heavy chain	gi|590561836|ref|XP_007008924.1|//Clathrin, heavy chain isoform 1 [Theobroma cacao]
9.08E-65	NA	NA	NA	K14306//nuclear pore complex protein Nup62	gi|359495964|ref|XP_002267137.2|//PREDICTED: uncharacterized protein LOC100266068 [Vitis vinifera]
2.78E-64	NA	GO:0000166//nucleotide binding;GO:0003916//DNA topoisomerase activity	GO:0006259//DNA metabolic process	K03164//DNA topoisomerase II [EC:5.99.1.3]	gi|720077984|ref|XP_010241214.1|//PREDICTED: DNA topoisomerase 2-like isoform X2 [Nelumbo nucifera]
3.31E-64	NA	GO:0000166//nucleotide binding	NA	K10357//myosin V	gi|567919980|ref|XP_006451996.1|//hypothetical protein CICLE_v10007271mg [Citrus clementina]
6.41E-64	GO:0005643//nuclear pore	GO:0005487//nucleocytoplasmic transporter activity	NA	K14303//nuclear pore complex protein Nup160	gi|296087564|emb|CBI34153.3|//unnamed protein product [Vitis vinifera]
1.52E-63	NA	NA	NA	NA	NA
3.83E-62	NA	GO:0005509//calcium ion binding	NA	K02183//calmodulin	gi|595807593|ref|XP_007202636.1|//hypothetical protein PRUPE_ppa012070mg [Prunus persica]
1.00E-59	GO:0016021//integral component of membrane	GO:0005524//ATP binding;GO:0042626//ATPase activity, coupled to transmembrane movement of substances	GO:0055085//transmembrane transport	K05666//ATP-binding cassette, subfamily C (CFTR/MRP), member 2	gi|699262790|gb|AIU41637.1|//ABC transporter family protein [Hevea brasiliensis]
4.94E-59	GO:0016459//myosin complex	GO:0003779//actin binding;GO:0005524//ATP binding;GO:0003774//motor activity	NA	K10357//myosin V	gi|255546055|ref|XP_002514087.1|//myosin XI, putative [Ricinus communis]
1.64E-58	GO:0070971//endoplasmic reticulum exit site;GO:0005634//nucleus;GO:0019898//extrinsic component of membrane;GO:0005829//cytosol;GO:0005886//plasma membrane	GO:0005089//Rho guanyl-nucleotide exchange factor activity	GO:0008064//regulation of actin polymerization or depolymerization;GO:0009958//positive gravitropism;GO:0007264//small GTPase mediated signal transduction;GO:0010928//regulation of auxin mediated signaling pathway;GO:0043547//positive regulation of GTPase activity;GO:0008360//regulation of cell shape;GO:0016192//vesicle-mediated transport	NA	gi|225444565|ref|XP_002277136.1|//PREDICTED: dedicator of cytokinesis protein 11 [Vitis vinifera]
5.28E-58	NA	GO:0016853//isomerase activity	GO:0005975//carbohydrate metabolic process	K01785//aldose 1-epimerase [EC:5.1.3.3]	gi|590728934|ref|XP_007099714.1|//Galactose mutarotase-like superfamily protein, putative [Theobroma cacao]
1.86E-56	GO:0016020//membrane	NA	NA	K10352//myosin heavy chain;K09291//nucleoprotein TPR	gi|590594096|ref|XP_007017758.1|//Uncharacterized protein isoform 4 [Theobroma cacao]
2.88E-56	GO:0009570//chloroplast stroma	GO:0046872//metal ion binding;GO:0016041//glutamate synthase (ferredoxin) activity;GO:0051538//3 iron, 4 sulfur cluster binding	GO:0006541//glutamine metabolic process;GO:0097054//L-glutamate biosynthetic process;GO:0008652//cellular amino acid biosynthetic process;GO:0055114//oxidation-reduction process	K00284//glutamate synthase (ferredoxin) [EC:1.4.7.1]	gi|401808265|gb|AFQ20793.1|//glutamate synthase [Beta vulgaris]
2.44E-54	GO:0016020//membrane	NA	NA	NA	gi|297739426|emb|CBI29608.3|//unnamed protein product [Vitis vinifera]
9.21E-53	NA	NA	NA	K02937//large subunit ribosomal protein L7e	gi|294438964|gb|ACV83305.2|//hypothetical protein UP-4 [Dimocarpus longan]
9.84E-53	NA	GO:0043022//ribosome binding;GO:0003743//translation initiation factor activity;GO:0003746//translation elongation factor activity	GO:0006413//translational initiation;GO:0006452//translational frameshifting;GO:0045905//positive regulation of translational termination;GO:0045901//positive regulation of translational elongation	K03263//translation initiation factor 5A	gi|657384748|gb|AES96905.2|//eukaryotic translation initiation factor 5A4 [Medicago truncatula]

Biological Process	Kegg Orthology	Nr Description
NA	NA	gi|702327324|ref|XP_010053778.1|//PREDICTED: protein CURVATURE THYLAKOID 1A, chloroplastic [Eucalyptus grandis]
GO:0055114//oxidation-reduction process	K05579//NAD(P)H-quinone oxidoreductase subunit H [EC:1.6.5.3]	gi|357467749|ref|XP_003604159.1|//NAD(P)H-quinone oxidoreductase subunit H [Medicago truncatula]
NA	K09487//heat shock protein 90kDa beta	gi|698490000|ref|XP_009791518.1|//PREDICTED: endoplasmin homolog [Nicotiana sylvestris]
GO:0032012//regulation of ARF protein signal transduction;GO:0043547//positive regulation of GTPase activity	K18442//brefeldin A-inhibited guanine nucleotide-exchange protein	gi|225428344|ref|XP_002280001.1|//PREDICTED: brefeldin A-inhibited guanine nucleotide-exchange protein 2-like [Vitis vinifera]
GO:0006633//fatty acid biosynthetic process;GO:0055114//oxidation-reduction process;GO:0031408//oxylipin biosynthetic process	K00454//lipoxygenase [EC:1.13.11.12]	gi|326418056|gb|ADZ73653.1|//putative 13-lipoxygenase [Capsicum annuum]
NA	NA	gi|40287496|gb|AAR83862.1|//elicitor-inducible protein EIG-J7 [Capsicum annuum]
GO:0016567//protein ubiquitination	K10592//E3 ubiquitin-protein ligase HUWE1 [EC:2.3.2.26]	gi|590605394|ref|XP_007020478.1|//E3 ubiquitin protein ligase upl2, putative isoform 3 [Theobroma cacao]
GO:0016567//protein ubiquitination	K10592//E3 ubiquitin-protein ligase HUWE1 [EC:2.3.2.26]	gi|359492508|ref|XP_002283711.2|//PREDICTED: E3 ubiquitin-protein ligase UPL2-like [Vitis vinifera]
GO:0006694//steroid biosynthetic process;GO:0055114//oxidation-reduction process	K09753//cinnamoyl-CoA reductase [EC:1.2.1.44]	gi|703083360|ref|XP_010092193.1|//Bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase [Morus notabilis]
GO:0055114//oxidation-reduction process	K05280//flavonoid 3'-monooxygenase [EC:1.14.13.21]	gi|356968422|gb|AET43292.1|//CYP76AD3 [Mirabilis jalapa]
GO:0055114//oxidation-reduction process	K05280//flavonoid 3'-monooxygenase [EC:1.14.13.21]	gi|356968422|gb|AET43292.1|//CYP76AD3 [Mirabilis jalapa]
GO:0055114//oxidation-reduction process	K00517;K05280//flavonoid 3'-monooxygenase [EC:1.14.13.21]	gi|356968422|gb|AET43292.1|//CYP76AD3 [Mirabilis jalapa]
GO:0006694//steroid biosynthetic process;GO:0055114//oxidation-reduction process	K09753//cinnamoyl-CoA reductase [EC:1.2.1.44]	gi|703083360|ref|XP_010092193.1|//Bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase [Morus notabilis]
GO:0009735//response to cytokinin;GO:0048589//developmental growth;GO:0006537//glutamate biosynthetic process;GO:0055114//oxidation-reduction process;GO:0019676//ammonia assimilation cycle	K00264//glutamate synthase (NADPH/NADH) [EC:1.4.1.13 1.4.1.14]	gi|643706019|gb|KDP22151.1|//hypothetical protein JCGZ_25982 [Jatropha curcas]
GO:0006412//translation	K02930//large subunit ribosomal protein L4e	gi|703115170|ref|XP_010100833.1|//60S ribosomal protein L4 [Morus notabilis]
NA	K01051//pectinesterase [EC:3.1.1.11]	gi|661890295|emb|CDP05792.1|//unnamed protein product [Coffea canephora]
NA	K14310//nuclear pore complex protein Nup205	gi|297738947|emb|CBI28192.3|//unnamed protein product [Vitis vinifera]
NA	NA	NA
GO:0006020//inositol metabolic process;GO:0032259//methylation	K13066//caffeic acid 3-O-methyltransferase [EC:2.1.1.68]	gi|1170555|sp|P45986.1|IMT1_MESCR//RecName: Full=Inositol 4-methyltransferase [Mesembryanthemum crystallinum]
GO:0006508//proteolysis;GO:0051301//cell division	K03798//cell division protease FtsH [EC:3.4.24.-]	gi|302141673|emb|CBI18876.3|//unnamed protein product [Vitis vinifera]
NA	K01188//beta-glucosidase [EC:3.2.1.21]	gi|703065069|ref|XP_010087358.1|//Beta-glucosidase 12 [Morus notabilis]

Biological Process	Kegg Orthology	Nr Description
GO:0006508//proteolysis	K01304//pyroglutamyl-peptidase [EC:3.4.19.3]	gi|641819523|gb|KDO39792.1|//hypothetical protein CISIN_1g024368mg [Citrus sinensis]
NA	K13195//cold-inducible RNA-binding protein;K12897//transformer-2 protein	gi|672173319|ref|XP_008806788.1|//PREDICTED: glycine-rich RNA-binding protein 3, mitochondrial [Phoenix dactylifera]
GO:0036258//multivesicular body assembly;GO:0070676//intralumenal vesicle formation	K00921//1-phosphatidylinositol-3-phosphate 5-kinase [EC:2.7.1.150]	gi|596141459|ref|XP_007222458.1|//hypothetical protein PRUPE_ppa003456mg [Prunus persica]
NA	K00454//lipoxygenase [EC:1.13.11.12]	gi|697160377|ref|XP_009588961.1|//PREDICTED: linoleate 13S-lipoxygenase 2-1, chloroplastic-like [Nicotiana tomentosiformis]
GO:0006886//intracellular protein transport;GO:0016192//vesicle-mediated transport	K04646//clathrin heavy chain	gi|590561836|ref|XP_007008924.1|//Clathrin, heavy chain isoform 1 [Theobroma cacao]
NA	K14306//nuclear pore complex protein Nup62	gi|359495964|ref|XP_002267137.2|//PREDICTED: uncharacterized protein LOC100266068 [Vitis vinifera]
GO:0006259//DNA metabolic process	K03164//DNA topoisomerase II [EC:5.99.1.3]	gi|720077984|ref|XP_010241214.1|//PREDICTED: DNA topoisomerase 2-like isoform X2 [Nelumbo nucifera]
NA	K10357//myosin V	gi|567919980|ref|XP_006451996.1|//hypothetical protein CICLE_v10007271mg [Citrus clementina]
NA	K14303//nuclear pore complex protein Nup160	gi|296087564|emb|CBI34153.3|//unnamed protein product [Vitis vinifera]
NA	NA	NA
NA	K02183//calmodulin	gi|595807593|ref|XP_007202636.1|//hypothetical protein PRUPE_ppa012070mg [Prunus persica]
GO:0055085//transmembrane transport	K05666//ATP-binding cassette, subfamily C (CFTR/MRP), member 2	gi|699262790|gb|AIU41637.1|//ABC transporter family protein [Hevea brasiliensis]
NA	K10357//myosin V	gi|255546055|ref|XP_002514087.1|//myosin XI, putative [Ricinus communis]
GO:0008064//regulation of actin polymerization or depolymerization;GO:0009958//positive gravitropism;GO:0007264//small GTPase mediated signal transduction;GO:0010928//regulation of auxin mediated signaling pathway;GO:0043547//positive regulation of GTPase activity;GO:0008360//regulation of cell shape;GO:0016192//vesicle-mediated transport	NA	gi|225444565|ref|XP_002277136.1|//PREDICTED: dedicator of cytokinesis protein 11 [Vitis vinifera]
GO:0005975//carbohydrate metabolic process	K01785//aldose 1-epimerase [EC:5.1.3.3]	gi|590728934|ref|XP_007099714.1|//Galactose mutarotase-like superfamily protein, putative [Theobroma cacao]
NA	K10352//myosin heavy chain;K09291//nucleoprotein TPR	gi|590594096|ref|XP_007017758.1|//Uncharacterized protein isoform 4 [Theobroma cacao]
GO:0006541//glutamine metabolic process;GO:0097054//L-glutamate biosynthetic process;GO:0008652//cellular amino acid biosynthetic process;GO:0055114//oxidation-reduction process	K00284//glutamate synthase (ferredoxin) [EC:1.4.7.1]	gi|401808265|gb|AFQ20793.1|//glutamate synthase [Beta vulgaris]
NA	NA	gi|297739426|emb|CBI29608.3|//unnamed protein product [Vitis vinifera]
NA	K02937//large subunit ribosomal protein L7e	gi|294438964|gb|ACV83305.2|//hypothetical protein UP-4 [Dimocarpus longan]
GO:0006413//translational initiation;GO:0006452//translational frameshifting;GO:0045905//positive regulation of translational termination;GO:0045901//positive regulation of translational elongation	K03263//translation initiation factor 5A	gi|657384748|gb|AES96905.2|//eukaryotic translation initiation factor 5A4 [Medicago truncatula]
NA	K09291//nucleoprotein TPR	gi|567903030|ref|XP_006444003.1|//hypothetical protein CICLE_v10018459mg [Citrus clementina]
GO:0006979//response to oxidative stress;GO:0055114//oxidation-reduction process	K00432//glutathione peroxidase [EC:1.11.1.9]	gi|460397927|ref|XP_004244516.1|//PREDICTED: probable phospholipid hydroperoxide glutathione peroxidase [Solanum lycopersicum]
GO:0006810//transport;GO:0009653//anatomical structure morphogenesis;GO:0006970//response to osmotic stress;GO:0009888//tissue development;GO:0048583//regulation of response to stimulus;GO:0098542//defense response to other organism;GO:0009755//hormone-mediated signaling pathway;GO:0009617//response to bacterium	K14513//ethylene-insensitive protein 2	gi|225440009|ref|XP_002276399.1|//PREDICTED: ethylene-insensitive protein 2-like [Vitis vinifera]

Biological Process	Kegg Orthology	Nr Description
GO:0016043//cellular component organization;GO:0016310//phosphorylation;GO:0046488//phosphatidylinositol metabolic process	K00921//1-phosphatidylinositol-3-phosphate 5-kinase [EC:2.7.1.150]	gi|720056901|ref|XP_010273819.1|//PREDICTED: 1-phosphatidylinositol-3-phosphate 5-kinase FAB1B [Nelumbo nucifera]
GO:0006886//intracellular protein transport;GO:0016192//vesicle-mediated transport	K04646//clathrin heavy chain	gi|590561836|ref|XP_007008924.1|//Clathrin, heavy chain isoform 1 [Theobroma cacao]
GO:0055085//transmembrane transport	K05658//ATP-binding cassette, subfamily B (MDR/TAP), member 1 [EC:3.6.3.44]	gi|698518797|ref|XP_009804265.1|//PREDICTED: ABC transporter B family member 6 isoform X1 [Nicotiana sylvestris]
NA	K12472//epidermal growth factor receptor substrate 15	gi|357509539|ref|XP_003625058.1|//Pathogen-induced calmodulin-binding protein [Medicago truncatula]
NA	K01785//aldose 1-epimerase [EC:5.1.3.3]	gi|672108302|ref|XP_008784468.1|//PREDICTED: aldose 1-epimerase-like [Phoenix dactylifera]

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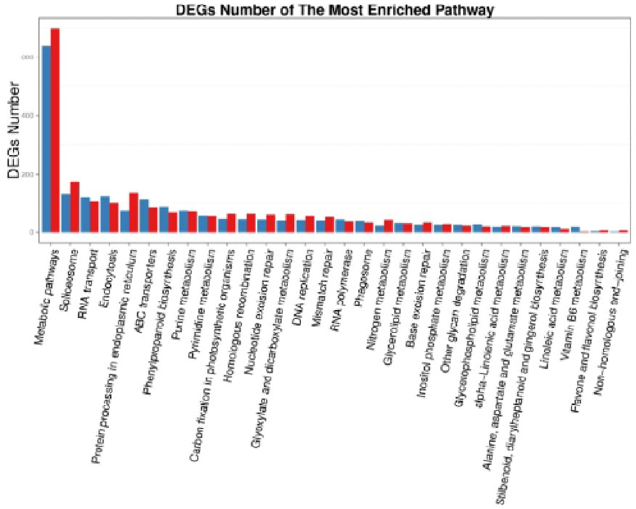
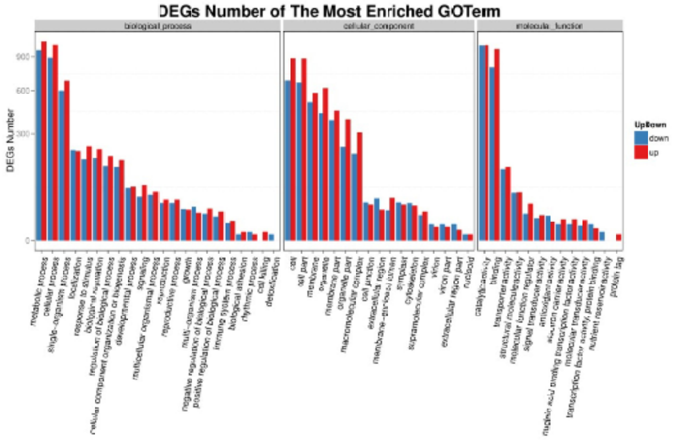


Fig. 4. Pathway functional enrichment result for up/down regulation genes.
X - axis represents the terms of Pathway. Y- axis represents the number of up/down regulation genes.



X-axis represents GO term. Y-axis represents the amount of up/down-regulated genes.
Fig. 5. GO classification of up-regulated and down-regulated genes.

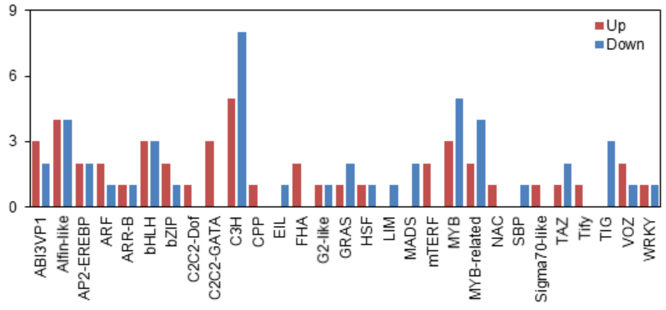


Fig. 6. DEGs classification on TF family.

Conclusion:

The obvious differences of transcriptomes between purple and white bracts of *Bougainvillea glabra* 'Mrs. Eva' mainly focus on a small proportion of the biological processes: the transcription of plant pigment and nitrogen metabolism pathways is strengthen in the purple bracts, while that of the stress response-related pathway is strengthen in the white bracts. In sum, these results facilitate shedding light on the molecular and biochemistry mechanisms underlying the bract coloration in *Bougainvillea*.

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