DIFFERENTIALLY EXPRESSED TRANSCRIPTS RELATED TO HEIGHT IN OIL PALM

INTAN NUR AINNI MOHAMED AZNI*; PARAMESWARI NAMASIVAYAM*; HO CHAI LING*; SHARIFAH SHAHRUL RABIAH SYED ALWEE and MOHAMAD ARIF ABD MANAF‡**

ABSTRACT

A subtractive cDNA library was constructed using MPOB Planting Series 1 (PS1) population to isolate differentially expressed transcripts associated with height increment in oil palm. After differential screening, 98 clones were identified to be potentially positive with cDNA inserts ranging from 250 to 1000 bp. A total of 123 sequences generated and low quality sequences ≤ 20 were eliminated using Phred program to assess the sequence quality and determine the accurate consensus sequence. Contig assembly by CAP3 program generated 51 contigs and four singletons (55 unigenes). Blast search showed 49 unique sequences had significant match to various plant species in the GeneBank database with E-value ≤ 1e-5, and four sequences showed no significant similarity. Gene ontology analysis output from Blast2GO program revealed that the sequences encoding for auxin responsive protein, circadian clock-associated protein1, zinc-finger protein and basic leucine zipper protein were potentially associated to dwarfism. The genes were identified based on their putative functions in regulating height in plants, particularly in growth hormone biosynthesis such as auxin (Aux/IAA), gibberellins (GA) and brassinosteroids (BR).

Keywords: differentially expressed transcripts, suppression subtractive hybridisation (SSH), dwarfism, auxin, gibberellins, brassinosteroids.

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INTRODUCTION

The effort towards developing dwarf palm populations with novel traits has great importance to the oil palm industry, mainly due to the high cost of harvesting fruits from tall palms. Dwarf palms are much easier to harvest and maintain. Reducing height increment in palm will bring positive effects on harvesting cost and significantly extend the economic cropping cycle. To date, many breeding trials have been designed to obtain superior genetic varieties particularly in generating dwarf palms with high oil yield. The Malaysian Palm Oil Board (MPOB) has conducted a series of trials using seeds from different producers to generate good quality palms, particularly low in height increment (Corley and Tinker, 2003). The Nigerian germplasm of MPOB Planting Series 1 (PS1) population used in this study was originated from crosses of Nigerian *dura*s and AVROS *pisifera*s. Sharma (1999) has reported good characteristics of this PS1 population with dwarf ideotype (population 12). Referring to the PS1 origin, the selection of Nigerian *dura*s collection was of great interest because of its low annual height increment (Rajanaidu and Jalani, 1994) and high bunch yields. The AVROS *pisifera*s, on the other hand, possess

* Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

** FELDA Biotechnology Centre, PT 23417, Lengkuk Technology, Persiaran Technology, Techpark@enstek, 71760 Bandar Enstek, Negeri Sembilan, Malaysia.

‡ Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.
E-mail: arifma@mpob.gov.my

high oil-yield, vigorous growth (Janick and Paull, 2008), precocious fruit bearing, thin shell and thick mesocarp. It was originated from the backcrossing of a well-known progenitor, SP540 *tenera* palm to the SP540 selves.

Being a potentially useful oil palm population with novel traits, PS1 is the best material to provide information on dwarfism. To date, the genes associated to height regulation have not been reported in the oil palm genome. Yet, no specific research to identify genes associated in oil palm dwarfism has been conducted. Besides oil palm, dwarf trait is also valuable in many agricultural plants because it provides a significant advantage to the industries. For example in cereal crops, the production of dwarf varieties become indispensible with the aims of increasing oil yields, improving lodging resistance, ease of harvesting and increased resistance to wind and rain (Kovi *et al*., 2011). Studies on dwarfing genes have been conducted in various plant species such as rice (Qiao and Zhao, 2011), *Arabidopsis thaliana* (Chung *et al*., 2010), barley (Gruszka *et al*., 2011) and tomato (Bishop *et al*., 1999).

Dwarfing genes are highly associated with the biosynthesis of plant growth hormones such as gibberellin (Kovi *et al*., 2011), auxin (De Grauwe *et al*., 2007) and brassinosteroids (BR) (Chung *et al*., 2010; Hossain *et al*., 2012). Gibberellin plays an important role in plant growth and development such as stem elongation, pollen tube growth, sex determination, seed germination, flower and fruit development, and the control of juvenility (George *et al*., 2008). Defective or blocking of signaling pathways of gibberellin biosynthesis by dwarfing agents or 'anti-gibberellins' such as onium compounds (chlormequat chloride, CCC and Amo1617), heterocyclic compounds (paclobutrazol, ancymidol and uniconazole), and acylhexanediones (daminozide) resulted in the dwarf or semi-dwarf stature of the plants (George *et al*., 2008).

Auxin, predominantly represented by indole-3-acetic acid (IAA), is the first discovered plant hormone which is responsible for plant growth and development such as induction of stem elongation, cell division and differentiation (Mano and Nemoto, 2012). Auxin is highly expressed in stems and flowers. The disruption of its biosynthetic pathways results in plant phenotypic defects. For example, the *vanishing tassel2(vt2)* gene of maize exhibits short stature, short inflorescence, defects in axillary meristem formation due to defective tryptamine (TAM) pathway within the IAA biosynthesis (Phillips *et al*., 2011). In *A. thaliana*, the repression of auxin-induced gene expression during the early stage of auxin biosynthesis lowers of auxin level and the short hypocotyl phenotype (Liscum and Reed, 2002). BR stimulates various aspects of plant morphogenesis such as seed germination, cell division and elongation, photosynthesis, development and growth during darkness, response to abiotic stress, senescence, and flowering and reproduction (Gruszka *et al*., 2011). BR-deficient mutants in various plant species such as *A. thaliana dwf1, cpd/dwf3, dwf4, dwf5, det2/dwf6* and *ste1/dwf7* (Chung *et al*., 2010), tomato *dwarf* (Bishop *et al*., 1999) and barley *HvDWARF* (Gruszka *et al*., 2011) have been investigated to elucidate the BR biosynthetic pathways.

In this study, suppression subtractive hybridisation (SSH) method was carried out to obtain the differentially expressed dwarfing genes in PS1 population. This approach is highly effective for the identification of rare messages between two distinct populations, where similar or closely-related genes are eliminated (Diatchenko *et al*., 1996). In SSH, the suppression PCR steps prevent undesirable amplification of unwanted genes. SSH has provided novel strategies for functional gene detection where the concentration of the high and low abundance cDNA were equalised, followed by suppression of unwanted genes (Diatchenko *et al*., 1996; Lukyanov *et al*., 2007). Diatchenko *et al*. (1996) also reported that SSH is able to generate clones that are approximately 90% different and reduce false positives. This method has been successfully used to isolate interesting genes in rice, wheat and maize.

The objective of this study is to identify differentially expressed genes which regulate oil palm dwarfism in the MPOB PS1 population.

MATERIALS AND METHODS

Plant Materials

The spear leaves of Frond 0 were obtained from the *Dura* x *Pisifera* (DxP) PS1, a MPOB breeding line at the MPOB-UKM joint project field in Bangi Selangor, Malaysia. Spear leaf tissue samples were collected from standard (409-1206) and dwarf (303-210) palms with a height increment of 40 cm yr^{-1} and 20 cm yr^{-1}, respectively. The leaf tissues were cleaned with distilled water and immediately frozen in liquid nitrogen and stored at -80°C until required.

RNA Isolation

Total RNA was extracted from 10 g of frozen spear leaf tissues with a modified method of Prescott and Martin (1987). The total RNA was cleaned using RNeasy Plant Mini Kit (Qiagen, USA) according to the manufacturer's instructions. The purified total RNA was electrophoresed in 1X TAE at 80 V to check the RNA integrity. Concentration

of total RNA was determined by Nanodrop ND-1000 spectrophotometer (Nano-Drop Technologies, USA).

Construction of cDNA Library

SSH was carried out with the PCR-Select cDNA Subtraction Kit (Clontech, Mountain View, CA, USA) according to the manufacturer's instructions. In this protocol, total RNA was converted into cDNA. The cDNA of the dwarf palms was designated as 'tester', while cDNA from the standard palm as 'driver'. The conversion of the control poly A^+ RNA (from human skeletal muscle) provided with the kit was performed alongside with the 'tester' and 'driver' to analyse the efficiency of SSH. The tester, driver and control cDNA were separately digested with *Rsa*I to obtain blunt-ended cDNA fragments. The tester cDNA was subdivided into two potions and each tester was ligated with different adaptors (adaptor 1 and adaptor 2R). The adaptor-ligated tester cDNA were used in two rounds of hybridisation and polymerase chain reaction (PCR) step. In the first hybridisation, an excess cDNA driver was hybridised at 68°C for 8 hr with each adaptor-ligated tester cDNA. In the second hybridisation, the two groups of samples from the first hybridisation were hybridised with freshly denatured driver cDNA at 68°C overnight.

The primary PCR was carried out in 25 µl reaction mixture containing 3 µl of diluted cDNA (*i.e.*, each subtracted sample from hybridisation procedure and the corresponding diluted unsubtracted tester control), 17.5 µl of sterile water, 1X PCR reaction buffer, 0.2 mM dNTP mix, 0.4 µM PCR primer 1 (5'-CTAATACGACTCACTATAGGGC-3'), that were complementary to adaptor 1 (forward: 5'-CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGT-3' and reverse: 3'-G GCCCGTCCA-5'), and adaptor 2R (forward: 5'-CTAATACGACTCACTATAGGGCAGCGTGGTCGCGGCCGAGGT-3' and reverse: 3'-GCCGGCTCCA-5') and 1X Advantage cDNA polymerase mix. The reaction mixture was overlaid with 50 µl of mineral oil and briefly centrifuged. The PCR steps were performed according to the following parameters: initial extension at 75°C for 5 min, polymerase activation at 94°C for 25 s and 27 cycles of denaturation at 94°C for 10 s, annealing at 66°C for 30 s, and extension at 72°C for 1.5 min. Then, the secondary PCR was carried out in 25 µl reaction mixture containing 3 µl of diluted primary PCR products, 16.5 µl of sterile water, 1X PCR of reaction buffer, 0.4 µM of nested PCR primer 1 (5'-TCGAGCGGCCGCCCGGGCAGGT-3'), 0.4 µM of nested PCR primer 2R (5'-AGCGTGGTCGCGGCCGAGGT-3'), 0.2 mM of dNTP mix and 1X of Advantage cDNA polymerase mix. The secondary PCR was carried out using the following programs: initial polymerase activation at 94°C for 20 s; 12 cycles of denaturation at 94°C for 10 s; annealing at 68°C for 30 s and extension at 72°C for 1.5 min.

The secondary PCR product harbouring differentially expressed gene transcripts was analysed by agarose gel electrophoresis. The subtracted cDNA were then cloned directly into the yT&A cloning vector (Yeastern Biotech, Taiwan) and transformed into ECOS™ 101 competent cell (Yeastern Biotech, Taiwan).

Screening of the Subtractive Clones

White colonies were randomly selected from the subtracted library grown on LB plates supplemented with 50 µg ml^{-1} ampicillin to screen for positive transformants. Single colony was inoculated into 7 ml LB broth supplemented with 50 µg ml^{-1} ampicillin and mixed well. The clones were grown overnight at 37°C with agitation in a shaker incubator. A total of 500 µl of the bacterial suspension culture were mixed with 50% glycerol stock in a microcentrifuge tube vigorously. The tubes were stored at -80°C for long-term storage. The remaining bacterial suspension culture was subjected to plasmid DNA isolation using QIAprep® Miniprep (QIAGEN, USA) in accordance to the manufacturer's instructions. Plasmids were digested with *Hin*dIII restriction enzyme to confirm the presence of the potential inserts.

Sequence Analysis

Plasmids harbouring putative transcripts were subjected to DNA sequencing by 1st BASE, Malaysia. Quality control of raw DNA sequences was performed using Phred program (Ewing *et al.*, 1998). Low quality sequences with a Phred value ≤ 20 were eliminated. The linearised vector, adapter sequences, and polyA and polyT were manually removed. These sequences were compared with the GenBank databases using the BLASTX program. Sequences with a BLASTX E-value ≤ 1e-05, and score 50 bits were considered as significant homology. The gene ontology (GO) terms by Blast2GO (Conesa and Götz, 2008) were classified manually according to its functional categorisation.

RESULTS AND DISCUSSION

Qualitative and Quantitative Analysis of RNA

The total RNA obtained was free from protein contaminants as indicated by the A_{260}/A_{280} ratio of 2.1. The A_{260}/A_{230} ratio of RNA (2.1 - 2.2) suggested that little or no polysaccharide and phenol contamination (Schultz *et al.*, 1994). Total RNA examined by electrophoresis showed distinct 28S and 18S rRNA bands, indicating that no or

little degradation occurred during the extraction (*Figure 1*). RNA quality analysis by Agilent's 2100 Bioanalyzer showed an RNA integrity number (RIN) of 6.8 – 7, indicating good RNA quality.

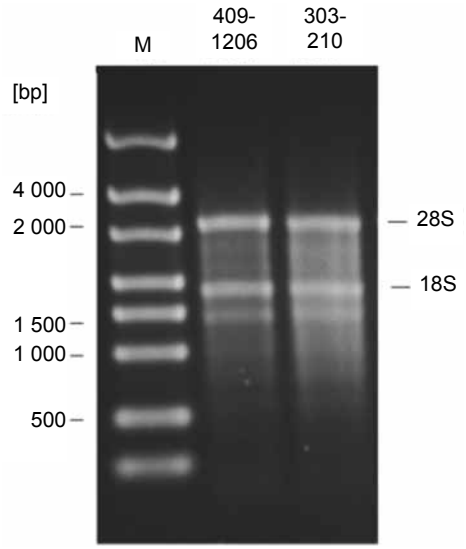

Figure 1. The total RNA isolated from spear leaves of 409-1206 and 303-210. M: RiboRuler™ high range RNA ladder (Fermentas).

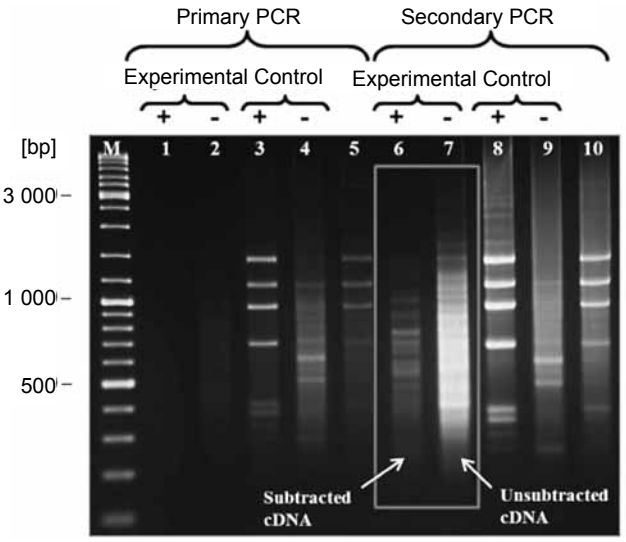

Figure 2. Analysis of polymerase chain reaction (PCR) products. Amplification of subtracted and unsubtracted cDNA from samples 409-1206, 303-210, control skeletal muscle and PCR control-subtracted cDNA. Primary (lanes 1-4) and secondary PCR (lanes 6-9) reactions were performed and analysed on a 2% (w/v) agarose gel. Lanes labelled '+' are subtracted and '-' are unsubtracted cDNA. Lanes 1 and 6: subtracted experimental cDNA. Lanes 2 and 7: unsubtracted experimental cDNA. Lanes 3 and 8: subtracted control skeletal muscle cDNA. Lanes 4 and 9: unsubtracted control skeletal muscle cDNA. Lanes 5 and 10: PCR control-subtracted cDNA. M: GeneRuler DNA Ladder Mix.

Isolation of Differentially Expressed Gene Transcripts

In this study, the total RNA was used as starting material without having to isolate mRNA because the cDNA library can be generated by using only a small amount of total RNA (Lukyanov *et al*., 2007). In SSH, in order to identify the potential candidate genes involved in dwarfism, cDNA from standard 409-1206 and dwarf 303-210 palms were compared. SSH eliminates similar genes that were abundantly expressed in both 'driver' and 'tester', while keeping only the unique cDNA transcripts (Diatchenko *et al*., 1996). The primary PCR products from the experimental SSH subtraction appeared as a smear from 300 to 1500 bp. The experimental secondary PCR products produced a few distinct bands suggesting that the abundance of identical genes had been eliminated. The secondary PCR products of subtracted and unsubtracted cDNA showed differences in their amplification patterns, indicating a successful subtraction. This was also supported by the result shown on the subtracted control PCR products where the banding pattern was clear and distinct as compared to the unsubtracted PCR products (*Figure 2*).

A total of 123 white colonies were randomly selected for screening of putative clones harboring inserts. As described by Lukyanov *et al*. (2007), SSH may also produce false positives, but at very low levels. In order to verify the putatively transformed clones containing inserts, restriction digestion using *Hin*dIII enzyme was carried out. In this study, the restriction enzyme analysis revealed that only 98 potential clones contain inserts ranging from 250 to 1200 bp (*Figure 3*).

Sequence Analysis

Ninety-eight potential clones were sent to 1st BASE, Malaysia for single pass DNA sequencing. The clones generated 186 sequences from both directions (forward and reverse). After sequence clean-up, 142 high quality sequences were further subjected to contig assembly. A total of 55 unigenes (51 contigs and four singletons) were generated. Contig sequences and singletons were compared to those in the non-redundant protein database in GenBank using BLASTX program with a cut-off E-value ≤ 1e-5 and score of 50 bits. Forty-eight unique sequences had significant matches. Ten sequences were highly similar to plant proteins in *Elaeis guineensis*, seven to *Vitis vinifera*, five to *Oryza sativa Japonica Group*, four to *Glycine max*, four to *Zea mays*, and 18 to other plant species.

Blast2GO assigned the 53 unique genes to 17 groups of biological processes, including protein metabolism, protein synthesis, protein binding, ATP binding, transporter activity, heat shock protein binding, transferase activity, DNA binding, signal transduction, transcription factor activity, response to biotic and abiotic stimulus, photosynthesis, growth factor activity, carbohydrate metabolic

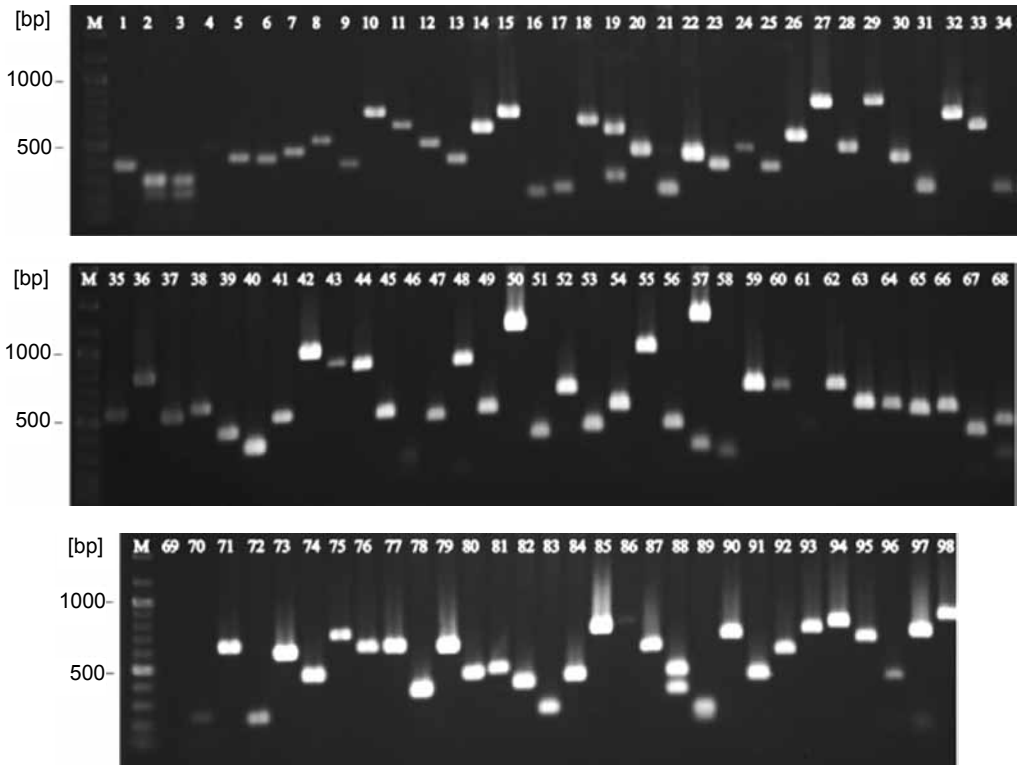

Figure 3. The inserts of 98 randomly selected clones in subtracted library. The putatively transformed clones carry inserts ranging from 250 to 1200 bp.

process, unknown function and no significant similarity (*Figure 4*). Nine genes were found to have unknown functions, and four genes had no significant similarity with known proteins or genes at the threshold E-value. The list of differentially expressed genes in dwarf oil palms is summarised in *Table 1*.

Selection of Candidate Genes that Regulate Oil Palm Dwarfism

Related dwarfing gene transcripts were identified based on their putative functions using Blast2GO as a reference. A few of these potential candidate dwarfing genes were identified to be involved in auxin, gibberellin and BRs biosynthetic pathways. These include circadian clock-associated FKF1 (Contig34), auxin-responsive protein (Contig38), zinc-finger protein, partial (Contig30 and Contig33), homeodomain-leucine zipper transcription factor TaHDZipI-1 (Contig41), and basic leucine zipper and W2 domain-containing protein (PS1_2_123R).

Sequence analysis by Blast2GO, auxin-responsive protein is classified into signal transduction process which mediates the environmental stimuli, and generates responses such as changes in gene expression. Based on various research reports, auxin-responsive proteins were involved in the regulation of auxin biosynthesis. According to Liscum and Reed (2002), Aux/IAA represses the synthesis of auxin at the early stage of auxin biosynthesis in *A. thaliana* mutant. The interaction of Aux/IAA with ARF results in the formation on heterodimers which then alter the auxin biosynthetic mechanism, and lower the auxin concentration.

Another candidate dwarfing gene is the circadian clock protein, an endogenous biological timer which is involved in signal transduction process. The gene controls the rhythmic processes including the leaf movement, stomata opening, transcription and hypocotyl elongation (De Grauwe *et al*., 2006). Circadian clock-associated1 (CCA1) acts to control the hypocotyl elongation in *A. thaliana* (Miyata *et al*., 2011). Plants overexpressing the circadian clock-associated1 have been reported to exhibit long hypocotyls, whereas the loss-of-function alleles cause a shortening in plant (Miyata *et al*., 2011). In addition, the interaction between CCA1 with late elongated hypocotyl (LHY) in evening elements exposure results in the repression of hypocotyl elongation in *A. thaliana* (De Grauwe *et al*., 2006).

Zinc-finger protein, a member of short internode (SHI) gene family based on The *Arabidopsis* Information Resource (TAIR) database, has a distinctive function to promote gynoecium, stamen and leaf development in *A. thaliana.* The gene was found as a negative regulator of gibberellin signaling, resulting in decreased internode length (Sun and Gubler, 2004). In this study, the gene was annotated to be involved in response to abiotic stimulus where

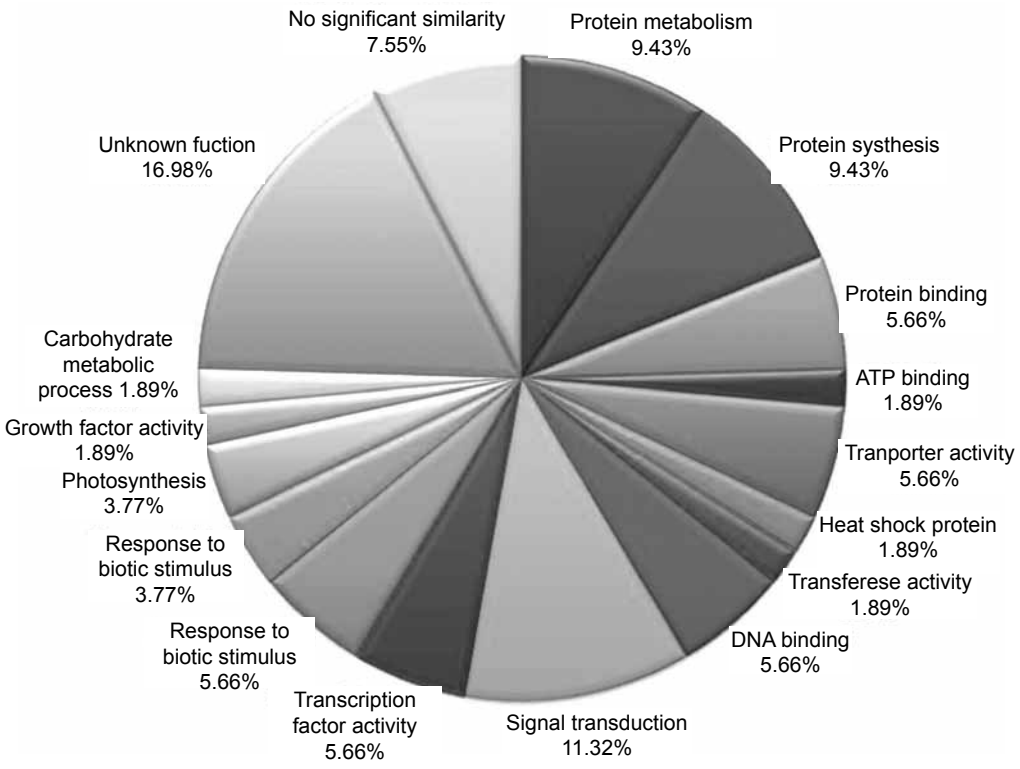

Figure 4. Biological process classification of the 53 unique genes extracted from subtracted library based on classification by Blast2GO.

TABLE 1. LIST OF DIFFERENTIALLY EXPRESSED GENES IN DWARF OIL PALMS CLASSIFIED BY THEIR PUTATIVE FUNCTIONS

Contig ID	Size (bp)	Accession No.[a]	Annotation[b]	E-value[c]	Identity (%)
Protein Metabolism (5)					
Contig16	539	AAG59584.1	pathogen-inducible alpha-dioxygenase [*Nicotiana attenuata*]	6e-72	76
Contig26	971	ACF17638.1	putative ketol-acid reductoisomerase [*Capsicum annuum*]	4e-170	90
Contig27	459	BAA11136.1	phospholipase D [*Oryza sativa Japonica* Group]	2e-88	89
Contig39	1 053	NP_001149903.1	protochlorophyllide reductase B [*Zea mays*]	2e-171	77
Contig46	606	BAD94991.1	cytoplasmic aconitate hydratase [*Arabidopsis thaliana*]	1e-55	88
Protein Synthesis (5)					
Contig2	503	BAA96068.1	60S ribosomal protein L27a [*Panax ginseng*]	1e-36	77
Contig8	361	XP_002274407.1	60S ribosomal protein L12 [*Vitis vinifera*]	9e-74	94
Contig10	321	XP_002285833.1	30S ribosomal protein S13, chloroplastic [*Vitis vinifera*]	2e-49	88
Contig21	416	ACF06450.1	ribosomal protein L10 [*Elaeis guineensis*]	4e-74	98
Contig43	567	ACF06464.1	ribosomal protein L32 [*Elaeis guineensis*]	2e-52	100
Protein Binding (3)					
Contig3	317	AEZ00871.1	putative 14-3-3 regulatory [*Elaeis guineensis*]	1e-13	87
Contig22	717	ACF06510.1	fibre protein Fb2 [*Elaeis guineensis*]	3e-89	99
Contig25	716	XP_003633689.1	peptidyl-prolyl *cis-trans* isomerase CYP19-3-like [*Vitis vinifera*]	3e-95	79
ATP Binding (1)					
Contig6	840	XP_002284518.1	ATP-dependent Clp protease ATP-binding subunit clpA homolog CD4A, chloroplastic-like [*Vitis vinifera*]	0.0	96
Transporter Activity (3)					
Contig9	411	XP_002454386.1	protein translocase/ protein transporter [*Zea mays*]	2e-57	83
Contig15	659	XP_002269591.1	PREDICTED: cation/H(+) antiporter 20-like [*Vitis vinifera*]	1e-60	61
Contig35	463	CCJ47269.1	putative proton-dependent oligopeptide or low-affinity nitrate transporter, partial [*Hordeum vulgare subsp. vulgare*]	4e-59	66
Heat Shock Protein Binding (1)					
Contig11	511	ABY52936.1	DnaJ family heat shock protein [*Oryza sativa Japonica* Group]	1e-92	77

TABLE 1. LIST OF DIFFERENTIALLY EXPRESSED GENES IN DWARF OIL PALMS CLASSIFIED BY THEIR PUTATIVE FUNCTIONS (continued)

Contig ID	Size (bp)	Accession No.[a]	Annotation[b]	E-value[c]	Identity (%)
Transferase Activity (3)					
Contig12	749	AEQ94124.1	putative O-methyltransferase [*Elaeis guineensis*]	1e-103	85
Contig41[d]	479	ABC86566.1	homeodomain-leucine zipper transcription factor TaHDZip-1 [*Triticum aestivum*]	6e-37	61
PS1_2_123R[d]	390	XP_003624182.1	basic leucine zipper and W2 domain-containing protein [*Medicago truncatula*]	5e-17	66
DNA Binding (3)					
Contig5	575	XP_003626874.1	hypothetical protein MTR_8g011490 [*Medicago truncatula*]	3e-13	37
Contig14	748	XP_003577287.1	PREDICTED: cohesin subunit SA-1-like [*Brachypodium distachyon*]	2e-129	75
Contig50	725	NP_001060521.1	nucleoid DNA-binding-like protein [*Oryza sativa Japonica* Group]	7e-50	59
Signal Transduction (6)					
Contig18	515	XP_002533880.1	Root phototropism protein, putative [*Ricinus communis*]	2e-26	67
Contig20	324	XP_002464201.1	hypothetical protein SORBIDRAFT_01g014000 [*Sorghum bicolor*]	3e-21	80
Contig34[d]	1 238	NP_001235886.1	circadian clock-associated FKF1 [*Glycine max*]	0.0	80
Contig37	302	AEQ94161.1	ADP-ribosylation factor [*Elaeis guineensis*]	1e-64	99
Contig38[d]	522	AEI70506.1	auxin-responsive protein [*Gossypium hirsutum*]	3e-28	95
Contig47	604	XP_002269380.1	PREDICTED: root phototropism protein 3 [*Vitis vinifera*]	5e-114	82
Transcription Factor Activity (1)					
Contig19	504	XP_003528761.1	PREDICTED: transcription factor bHLH70-like [*Glycine max*]	1e-05	38
Response to Abiotic Stimulus (3)					
Contig30[d]	323	AFS65093.1	zinc-finger protein, partial [*Elaeis guineensis*]	3e-29	100
Contig33[d]	341	AFS65093.1	zinc-finger protein, partial [*Elaeis guineensis*]	2e-09	65
Contig45	323	NP_001105283.1	INDETERMINATE-related protein 9 [*Zea mays*]	2e-11	55
Response to Biotic Stimulus (2)					
PS1_2_120R	284	AFS65102.1	CCR4-associated factor 1-related protein [*Elaeis guineensis*]	1e-32	100
Contig42	386	BAH79622.1	Soyabean dwarf virus resistance protein [*Glycine max*]	8e-36	87
Photosynthesis (2)					
Contig31	372	AAO45885.1	chlorophyll a/b-binding protein precursor [*Citrus limon*]	2e-84	100
Contig36	383	ABF74606.1	chloroplast chlorophyll a/b-binding protein [*Agave tequilana*]	1e-57	98
Growth Factor Activity (1)					
Contig44	502	ABF70025.1	phytosulfokine family protein [Musa acuminata]	2e-07	55
Carbohydrate Metabolic Process (1)					
Contig51	592	ACF06618.1	meloidogyne-induced giant cell protein-like protein [*Elaeis guineensis*]	1e-130	99
Unknown Function (9)					
Contig4	539	ACF79323.1	unknown [*Zea mays*]	9e-44	53
Contig23	388	XP_003536645.1	PREDICTED: ALG-2 interacting protein X-like [*Glycine max*]	2e-05	100
Contig28	342	ABK96748.1	unknown [*Populus trichocarpa* x *Populus deltoides*]	2e-26	73
Contig29	375	XP_002489117.1	hypothetical protein SORBIDRAFT_0057s002150 [*Sorghum bicolor*]	5e-50	89
Contig32	452	CAN66875.1	hypothetical protein VITISV_009275 [*Vitis vinifera*]	6e-32	90
Contig37	302	ACJ84889.1	unknown [*Medicago truncatula*]	1e-64	98
Contig40	806	NP_001051264.1	Os03g0748000 [*Oryza sativa Japonica* Group]	5e-107	65
Contig48	444	XP_002297967.1	predicted protein [*Populus trichocarpa*]	3e-43	87
Contig49	398	NP_001054235.1	Os04g0674000 [*Oryza sativa Japonica* Group]	7e-09	39
No Significant Similarity (4)					
Contig17	702		No significant similarity found		
Contig24	312		No significant similarity found		
PS1_2_4R	451		No significant similarity found		
PS1_2_124R	293		No significant similarity found		

Note: [a] GenBank accession number. All cDNA sequences were submitted to GenBank.
[b] Annotation of each SSH-contigs sequences was performed using Blast2GO program.
[c] The expect value (E) is the number of hits one can 'expect' to see by chance when searching in the GenBank database. The lower the E value, of the closer it is to zero, the more 'significant' the match is. The cut-off value used was 10-5.
[d] The candidate genes that regulate oil palm dwarfism.

the alteration of cell activity (movement, secretion, enzyme production and gene expression) was due to the response to non-living stimulus such as temperature, gravity, light, pH, osmotic and water.

Alonso *et al*. (2009) has reported that basic leucine zippers protein, bZIP53 acts to stimulate the transcription process in seed maturation in *A. thaliana*, and elicits phenotype alterations with dwarfism and delayed bolting in comparison with the wild-type plants. In tobacco, basic leucine zipper protein was identified to be one of the regulatory factors that controlled the GA biosynthesis, and thus was designated as a repressor of shoot growth (RSG) (Thomas *et al*., 2005). RSG gene which encodes for bZIP transcription factor acts to reduce the amount of endogenous GA, exhibits dominant-negative dwarf phenotypes with reduced GA_1 expression. The expression of RSG gene in transgenic tobacco specifically inhibits the stem internode growth.

Apart from the above, many research reports have revealed that these five differentially expressed dwarfing gene transcripts are involved in developing dwarf phenotype. These unique genes can be further investigated by quantitative real-time PCR (qRT-PCR) to validate the gene expression level in dwarf and standard palms.

CONCLUSION

In conclusion, potential candidate genes related to height in MPOB PSI population were identified. Five putative dwarfing genes transcripts were identified to be differentially expressed in this population such as auxin-responsive protein, circadian clock-associated FKF1, zinc-finger protein, homeodomain-leucine zipper transcription factor TaHDZipI-1, and basic leucine zipper and W2 domain-containing protein. Additional work needs to be carried out to elucidate their functions. These findings will provide helpful information for future research aiming to isolate differentially expressed genes related to dwarfism.

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