TRANSCRIPTOMIC ANALYSIS FOR TWO EGYPTIAN RICE CULTIVARS (*Oryza sativa*) UNDER DROUGHT STRESS

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Rice (*Oryza sativa* L.) is one of the most important crops around the world. In Egypt, rice is considered a strategic crop as essential traditional diets. It represents 22% of total cultivated area in Egypt and consumes around 20% of total water available for irrigation (Omar and Moussa, 2016). The cultivated rice area in Egypt was 1.424 million feddans with an average of 4 T fed^{-1}, and a total productivity 5.70 million tons over the past five years (RRTC, 2014).

Rice suffers from several abiotic stresses such as drought, salinity, high and low temperature and flooding that adversely affect crop yield (Hassan *et al.,* 2013). One third of rice cultivated area suffer from water shortage, and rice crop exposed to drought condition one or more time during growth stages (Abd Allah *et al.,* 2016).

Drought affects cell water potential, turgidity, membrane stability and photo-oxidation of chlorophyll leading to accumulation of reactive oxygen species (ROS), generated mostly in chloroplast, mitochondria and prexisome, causing oxidative stress (Lum *et al.,* 2014). ROS molecules such as singlet oxygen ($^1O_2$), superoxide anion radical (O_2^-), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) are highly active molecules that react with biological molecules such as lipid, carbohydrate, protein, chlorophyll and nucleic acid, unlimited oxidation of cellular component lead to cell death (Das and Roychoudhury, 2014). Therefore, drought stress is considered one of the most serious stresses to rice production where 50% of rice crop loss annually caused by drought (Moonmoon *et al.,* 2017).

In order to identify drought related genes, many molecular techniques like, differential display reverse transcription (DDRT) (Borah *et al.,* 2017) and quantitative real-time PCR (qRT-PCR) (Pabuayon *et al.,* 2016) have been used to measure and differentiate between levels of gene expression. Differential display reverse transcription (DDRT) is sensitive and effective technique in transcriptome analy-

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sis, compare several gene expression profiling and isolation of important genes (Borah *et al.,* 2017). This technique has been used previously with several scientific groups, Abou Ali *et al.,* (2010) who detected ABA-induced gene expression in *Vicia villosa*. In addition, Dang *et al.,* (2014) used the same technique in transcriptome sequencing data analysis of *Reaumuria trigyna*. Manjesh *et al.,* (2017) who used DDRT in the study of the gene expression of rice plants treated with *Pseudomonas fluorescens* strain at the reproductive stage under drought stress.

Many drought related genes recently discovered in rice to provide new insights into mechanisms regulated under this stress. For instance, *Ghd2* was down-regulated under drought condition (Liu *et. al.,* 2016), *RSOsPR10* (root-specific pathogenesis-related protein 10) expressed in cortical cell of rice (Tomokazu *et. al.*, 2016), *OsICE1* (rice inducer of CBF expression 1) up-regulated in rice roots (Chandera *et. al.,* 2018), *OsLG3* were found to play a role in rice grain length enhancement under stress (Xiong *et. al.,* 2018), *OsJMJ703* (JmjC-domain-containing (JmjC) protein, an important kind of histone demethylase in plants were expressed in various tissues of rice plants and down -regulated under stress condition (Zhu *et. al.,* 2018).

In the current study, there is a trail to understand the induced drought tolerance mechanisms in rice through isolation of some drought related genes from drought tolerant and drought sensitive rice cultivars.

MATERIALS AND METHODS

Plant materials

Two Egyptian rice cultivars (*Oryza sativa* L.), Giza 179 and Sakha 101 were obtained from the, Rice Department, Field Crops Research Institute (FCRI), Agriculture Research Center (ARC), Giza, Egypt.

1..1. Grains sterilization, germination and treatment

The grains were sterilized and germinated according to Abou Ali *et. al.,* (2019). Ten grains per jar for 9-12 days. For treatment, seedlings were transferred into solution 20% PEG (8000) for 24 hr Campo *et. al.,* (2012). The control seedlings were placed in sterilized d H_2O. The treated and control seedlings were harvested, and quick frozen in liquid nitrogen, then stored at -80°C until RNA extraction.

1..2. RNA extraction from plant material

Total RNA was extracted from the untreated and treated seedlings according to Chomczynski (1993), using the TriPure reagent (Roche Molecular Biochemicals, cat. No.1667165). The total RNA from each sample was examined by agarose gel electrophoresis 1%.

1..3. Differential display polymerase chain reaction (DD-PCR)

All DNA primers used in PCR reactions (Table 1) were purchased from Operon Molecular for Life (Germany).

The PCR reactions and programs

were carried out according to Radwan *et. al.,* (2015) in a Bio-RAD PCR instrument (USA).The extracted RNA was used to form the cDNA first strand. The cDNA reactions were carried out according to the manufacturer's protocol of M-MLV reverse transcriptase (200 units, cat. No. 0000113467, Promega, USA). Before constructing the differential display reactions, the cDNAs synthesis were checked firstly by PCR reactions on them using the *actin* (housekeeping gene).

The anchored primer used in PCR amplification was the same one used for RT. Different combination of anchored and arbitrary primer pairs were used. The different combinations were as follows:

Anchored primer $T_{11}A$ with arbitrary primer HAP3 and named (SAP3)
Anchored primer $T_{11}A$ with arbitrary primer HAP2 and named (SAP2)
Anchored primer $T_{11}A$ with arbitrary primer HAP26 and named (SAP26)
Anchored primer $T_{11}C$ with arbitrary primer HAP4 and named (SCP4)
Anchored primer $T_{11}C$ with arbitrary primer HAP28 and named (SCP28)
Where S is for shoot; A or C for the anchored primers is for anchored primer $T_{11}A$ or $T_{11}C$, respectively. While the numbers for the arbitrary primers.

The PCR products were loaded on 6% Urea polyarcylamide sequencing gel.

Isolation of DD-fragments from the gel and their Re-amplification

The DD-fragments were isolated and re-amplified according to Abou Ali *et. al.,* (2010) resuspended in 10 µl of dH_2O for reamplification by PCR using the same primer sets. The re-amplification conditions were as described previously DD-PCR reactions as follows: five µl DNA (from the isolated bands), 12.5 µl of PCR master mix, 1.0 µl of Anchored primer, 1.0 µl of Arbitrary primer and PCR reactions were completed to 25 µl. The re-amplified fragments were tested on 1.5% agrose gel.

1..4. Cloning of the re-amplified DD-fragments

The re-amplified fragments were cloned on pGEM-T vector system. Mini-preps of recombinant plasmid DNA and digestion reactions were carried out according to Sambrook *et. al.*, (1989). The EcoR1enzyme was used to release the insert in order to select positive transformants. The digestion reactions were loaded onto agarose gel (1.5%).

1..5. Sequencing and data analysis

The chain termination procedure (Sanger *et al.,* 1977) was performed using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, USA) with T7 and SP6 primers in conjunction with ABI PRISM (310 Genetic Analyzer). The nucleotide sequence or the deduced amino acid sequence of each clone was compared with DNA and protein sequences from various databases by means of the basic local alignment search tool (BLAST) in Gene-Bank (http://www.ncbi.nlm.nih.gov) (Altschul *et. al.,* 1997).

1..6. Quantification of the Targeted Genes by Real Time-PCR

Specific primers to amplify the tar-

geted gene were designed using primer blast and primer3 web online software for qPCR primer design tool. Primers were purchased from Operon Molecular for Life (Germany). The qRT-PCR was performed in AB7300 Real-Time PCR System® (Applied Biosystems, USA) using SYBR® Green PCR Kit from QIAGEN (Catalog no. 204141, QIAGEN, Germany). The fragments were amplified by using the primer pairs see (Table 2) in both G179 and Sk101 rice cultivars to compare the expression of the targeted genes in treated and untreated seedlings. PCR conditions as follows: one µl of cDNA first strand, 5 µl of PCR master mix, 0.3 µl of Forward primer (10 pmol), 0.3 µl of Reverse primer (10 pmol) and the RT PCR reactions were completed to 10 µl. The thermal cycler program was begun with activation step at 95°C for 15 min followed by sec step which consisting from 40 cycles of 94°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec then the melting curve step of 95°C for 15 sec, 60°C for 1 min and 95°C for 15 sec.

RESULTS AND DISCUSSION

Plants under drought stress displays several defense mechanisms to protect themselves from the damaging effects. Among these mechanisms increasing ROS scavenging molecules, enhancement of water uptake by deep, dense root systems, the minimization of water loss by stomatal closure and a reduction in leaf area and osmotic adjustment (OA) (Larkunthod *et. al .,* 2018).

In our previous study (Ibrahim *et al.,* 2019), several physiological parameters were analyzed on six Egyptian rice cultivars; from this analysis, two different cultivars were selected, the most tolerant cultivar (Giza 179) and the most sensitive one (Sakha 101). The two cultivars were subjected in the current study to differential display reverse transcription (DDRT) technique in order to isolate drought related gene(s).

1..7. RNA isolation and Differential display-PCR (DD-PCR)

Total RNA were isolated from treated and untreated samples of two different cultivars Giza 179 (G179) and Sakha 101 (Sk101). The isolated RNA were reverse transcribed to cDNA which was amplified by using combination from anchored ($T_{11}A$ and $T_{11}C$) and five random arbitrary primer (AP2, AP3, AP4, AP26 and AP28) in separate PCR reactions. The amplified products were analyzed on 6% urea polyacrylamide sequencing gels where $T_{11}A$/AP3 in Fig. (1), $T_{11}A$/AP2 and $T_{11}A$/AP26 in Fig. (2) and $T_{11}C$/AP4 and $T_{11}C$/AP28 in Fig. (3). Fifty-six and forty-seven DD-PCR fragments were differentially expressed under drought in G179 and Sk101, respectively.

1..8. Expression patterns of DD fragments

Based on the intensity of DD-fragments in treated and untreated plants in the two Egyptian rice cultivars, the expression profile was divided into two clusters (Table 3). Cluster 1 includes seven groups which indicated varying up-regulation patterns of drought related genes. Cluster 2 includes six groups which

indicated varying down-regulation patterns of drought related genes. All differentially expressed DD-fragment were separately excised from the gel, eluted. Seventeen DD-PCR fragments of them selected to be re-amplified by using the same primers. Agrose gel for the DD-PCR reamplified fragments were shown in Fig. (4).

1..9. Cloning of the re-amplified DD fragments in pGEM-T Easy vector

The selected re-amplified fragments were ligated into pGEM-T cloning vector and transformed into *E. coli* cells. The recombinant plasmids DNA were isolated and digested with *EcoRI* restriction enzyme (Fig. 5).

1..10. Sequencing of selected DD –PCR fragments

The DD-PCR fragments were sequenced. The fragments SAP3-3, SAP3-7, SAP3-8 and SAP3-19 had the same sequence, so they were subsequently considered as one fragment and were all designated as SAP3-3. Also, the fragments SCP4-1, SCP4-2 and SCP4-7 had the same sequence and were designated as SCP4-2. Tables (4, 5 and 6) represent the sequences of selected DD-fragments

1..11. Sequence analysis and gene expression of the DD-PCR fragments

The nucleotide sequence or the deduced amino acid sequence of each clone was compared with DNA and protein sequences from various databases by means of the basic local alignment search tool (BLAST) in Gene Bank (https://www.ncbi.nlm.nih.gov/) (Altschul *et. al*., 1997).

SAP3-2 fragment

The expression of SAP3-2 was found to be up regulated by drought treatment in G179 Egyptian rice cultivar, but did not appear in SaKha101 cultivar. Blast N showed significant homology with *Oryza sativa* japonica group chromosome 7 with e-value: 0, as well **as ribosome biogenesis** protein WDR12 homolog (LOC4343819) in *Oryza sativa* indica group (Yamamoto *et. al*., 2001) with e-value: 1e-43. Blast X showed significant homology with ribosome biogenesis protein WDR12 homolog in *Oryza sativa* Japonica Group with e-value: 3e-11. These results are important due to the relation between **ribosome biogenesis** and development of living creature (Zhao *et. al*., 2017). WDR12 is a member of the ribosomal gene family, which is interact with PES, BOP1 to form PeBoW complex. PeBoW complex were contributed in a many cellular processes, such as cell proliferation, signal transduction, gene regulation and apoptosis (Ahn *et. al.,* 2016).

Moreover, regulation of ribosome biogenesis is interlinked with the action of the phytohormone auxin (Huang *et. al.,* 2016). On another context, plants with affected ribosome biogenesis are less efficient in stress response and show severe phenotypes, e.g. under cold, drought, salt or metabolite stress (Maekawa *et. al.,* 2018). Moreover, WDR12 was inducted in tolerant cultivar (G179) and this might be back to auxin content in two cultivars un-

der drought stress (physiological results). Where G179 showed more pronounced auxin content than Sk101. Moreover, this result conflicted on continued cell division and cell elongation, thus, lead to better morphological criteria in tolerant cultivar than sensitive one. In addition, Deficient in DWR12 inhibits cell-cycle development. Consequently, defective effect on cell growth and proliferation occur (Ahn *et. al.,* 2016).

SAP3-3 fragment

The expression of SAP3-3 fragment was found to be up regulated by drought treatment in G179 Egyptian rice cultivar, but did not appear in SK101. BLAST N showed significant homology with *Oryza sativa* Japonica, chromosome 1, BAC clone: OJ1111_G1, with e value: 1e-120. Blast X showed significant homology **with chaperone protein DnaJ 15** in *Oryza sativa* Japonica Group two scores, the first score with e-value: 2e-04, as well **as putative ARG1** protein also in *Oryza sativa* Japonica Group with e-value: 5e-04. In general, chaperon are responsible mainly for protein folding, translocation and degradation in normal development as well as protein stabilization and refolding under unfavorable condition such as abiotic stresses (Salasmunoz *et. al.,* 2016). Chaperon included J protein or DnaJ proteins, which have important roles in plant immunity (Liu and Whitham, 2013), drought tolerance (Wang *et. al.,* 2014), in DNA replication and repair (Yamamoto *et. al.,* 2005), in development of chloroplast (Zhu *et. al.,* 2015) and play a role in cell division (Hu *et. al.,* 2015) Therefore, it was expected to have molecular chaperone with high expression in tolerant cultivar (G179) more than sensitive one (Sk101) in the current study under drought stress condition

SCP4-2 fragment

The expression of SCP4-2 was found to be up regulated by drought treatment in both G179 and SK101 cultivars. BLAST N showed significant homology with chromosome 6 in *Oryza sativa* Indica Group, with e value: 0.0. Blast X showed significant homology with conserved region RT_LTR: Reverse transcriptases (RTs) from **retrotransposons** and retroviruses (Accession number: cd01647) with e-value: 9.26e-03 and retrotransposon protein, putative, Ty3-gypsy subclass, expressed in *Oryza sativa* Japonica Group, with e value: 1e-98. These results well confirmed by quantitative real time PCR, in which Sk101 (sensitive) was showed (about two fold) increase comparing with G179 (tolerant cultivar) (Fig. 6).

SCP4-6 fragment

The expression of SCP4-6 fragment was found to be up regulated by drought treatment in SK101 but not appeared in G179. BLAST N showed significant homology with *oryza sativa* japonica chromosome 7 BAC clone: OSJNBa0077F02 with e value: 3e-103. Blast X showed significant homology with **transposon protein, putative**, CACTA, En/Spm sub-class in *Oryza sativa* Japonica Group with e value: 2e-30, as well as **hydroxyproline-rich** glycoprotein -like in *Oryza alta* with e value: 5e-25.

Transposable elements (TEs) are repetitive sequences of DNA, which have the ability to alter their position in a genome.

They are constituting large portion of eukaryotic genomes. For instance, 80% of maize genome is TEs. Because of their repetitive nature, TEs are responsible for fragmental gene movements, chromosomal rearrangements and for the evolution of gene regulation and function. Hence, TEs activity now is considered one of the main processes in genome evolution (Vitte *et. al.,* 2007). According to mechanism of transposition, TEs could be divide into two classes Class 1: depend upon "copy-and-paste" mechanism, in which RNA act as intermediate that reverse-transcribed into a copy of cDNA, which combined elsewhere in the genome and this known as retrotransposons. Class 2: depends upon "cut-and-paste" mechanism where intermediate is DNA, which mobilize directly from one cite to another through genome and this class known as transposons (Bourque *et al.,* 2018). In plants, the most widespread type of TE is Long Terminal Repeat (LTR) **retrotransposon** and considered the main constituents of large genomes of plants (Meyers *et. al.,* 2001). Comprehensive analyses from several assembled plant genomes has provided evidence that many genomes, such as 19% of peach (Verde *et. al.,* 2013), 62% of tomato (Sato *et. al.,* 2012), and 53% of potato (Xu *et. al.,* 2011), are composed of LTR-RTs. Furthermore, these elements might be responsible for wide genome expansions as well as their major role in the remarkable genome size variation and polyploidy (Piégu *et. al.,* 2006).

SCP4-3 fragment

The expression of SCP4-3 was found to be up regulated by drought treatment in SK101, but did not appear in G179 cultivar. BLAST N showed significant homology with **cytokinin riboside 5'-monophosphate phosphoribohydrolase** LOGL10 (LOC4348914), in *Oryza sativa* Japonica Group with e value: 0.0. Blast X showed significant homology with cytokinin riboside 5'-monophosphate phosphoribohydrolase LOGL10 in *Oryza sativa* Japonica Group with e value: 1e-32. Also showed conserved region named Lysine_decarboxylase, e value: 1.13e-07. **Cytokinin riboside 5'-monophosphate phosphoribohydrolase** is a cytokinin-activating enzyme controls the shoot meristem activity. Phosphoribohydrolase that converts inactive cytokinin nucleotides to the biologically active free-base forms. Moreover, it reacts specifically with cytokinin nucleoside 5'-monophosphates, but not with di-or triphosphate. This enzyme Expressed in many tissues of plant such as roots, leaves, stems, tiller buds, immature inflorescences and flowers. It expresses also in the upper part of shoot meristems, including axillary meristems, meristems of developing panicle and floral meristems. Moreover, cytokinins (CKs) have crucial impact on regulation of plant growth as well as on the stabilization of photosynthetic machinery during stress progression (Rivero *et. al.,* 2007). In the current, quantitative PCR showed slightly increase in active cytokinin under drought stress in both cultivars, which mean that both cultivars under treatment trial to increase active cytokinin in attempt to enhance efficiency of shoot system to overcome adverse effect of drought stress (Fig. 6).

SAP2-1 fragment

The expression of SAP2-1 fragment was found to be up regulated by drought treatment in Sk101 and not appear in G179 cultivar. BLAST N showed significant homology with **peroxisome biogenesis** protein 12 (LOC4348849) on *Oryza sativa* Japonica Group with e-value: 4e-63. Blast X showed conserved domain (mRING_PEX12) Modified RING finger found in peroxin-12 (PEX12) and similar proteins PEX12, also known as peroxisome assembly protein 12 or peroxisome assembly factor 3 (PAF-3). These results were confirmed by real time PCR, which showed that treated Sk101 (sensitive) was 6-fold more than G179 (tolerant) (Fig. 6). Peroxisomes are important cellular respiratory organelles which play vital roles in defense mechanisms under unfavorable conditions through their contents of enzymatic and non-enzymatic molecules. Where, they comprise anti-oxidant scavenge molecules such as glutathione and ascorbate as well as anti-oxidant enzymes, such as ascorbate peroxidase, catalase, dehydroascorbate reductas and glutathione reductase. Alterations in these enzyme activities are modulated under stress conditions (Ebeed *et. al.,* 2018). Moreover, they play a role in nitrogen metabolism, lipid mobilization *via* β-oxidation and the glyoxylate cycle, synthesis and metabolism of plant hormones and photorespiration (Hu *et. al.,* 2012).

Peroxisomes are very sensitive to cellular and environmental signals and responding by changes in number size and proteomic content. It responses to increased H_2O_2, Ozone and pathogen by enhancement its proliferation. Peroxins genes (PEX) are a group of protein factors involved in peroxisome biogenesis and peroxisome biogenesis protein 12 is one of this family (Ebeed *et. al.,* 2018). In this study, peroxisome biogenesis gene showed markedly up-regulation in sensitive cultivars under drought stress more than tolerant one. And this indicates the negative effect of drought condition and raises ROS levels in cells, also it considered a desperate attempt in response to this adverse effect.

SAP3-20 was found to be up-regulated in Sk101, while **SCP26-6** up-regulated in both cultivars. Both sequences showed significant homology with BAC clones in chromosomes number (9 and 12 respectively) in *Oryza sativa* (Katayose *et. al*., 2002). **SCP4-4** did not show any significant homology to known sequence neither in blast N nor blast X database.

SAP3-1 fragment

The expression of SAP3-1 was found to be up regulated by drought treatment in Egyptian G179 rice cultivar, but did not appear in SK101 rice cultivar. Blast N showed significant homology with *Oryza sativa* japonica chromosome 1 BAC clone OJ1111_G12. The e value was 3e-62. While blast X showed significant homology with hypothetical protein in *Oryza sativa* japonica OsJ_01630 with e value: 2e-06.

SCP4-8 fragment

The expression of SCP4-8 fragment was found to be down regulated by drought treatment in SK101 but not appeared in G179. BLAST N showed signif-

icant homology with chromosome 4 in *oryza sativa* japonica group, BAC clone: OSJNBa0053K19, with e value: 0.0. Amino acid sequence alignment SCP4-8 fragment showed significant homology with conserved region (Smc) Chromosome segregation **ATPase [Cell cycle control, cell division, chromosome partitioning],** (Yamamoto *et. al.*, 2001) Accession number COG1196, interval: 34-588, e value: 1.10e-14. In addition to hypothetical protein OsJ_16230 [*Oryza sativa* Japonica Group] with e value: 1e-124.

SCP28-2 fragment

The expression of SCP28-2 fragment was found to be down regulated by drought treatment in Sk101 and not appeared in G179 cultivar. BLAST N showed significant homology with chromosome 12 in *Oryza sativa* japonica group, BAC OSJNBa0036H15 with e value: 7e-152. Blast X showed significant homology with hypothetical protein *Oryza sativa* Japonica Group with e value: 0.039.

The blastX analysis for the sequences **SAP3-1, SCP4-8 and SCP28-2** showed homology with *Oryza sativa* BAC clones in chromosomes number (1, 4 and 12, respectively) which may be useful in determination of drought QTL in rice. While blast N analysis of the same fragments showed homology with *Oryza sativa* hypothetical proteins**. SAP3-1** found to be up-regulated in tolerant cultivar G179 and not appear in Sk101, it may be play a role in adaptation of plant under drought stress (Gojobori *et. al.,* 2002). While, **SCP4-8 and SCP28-2** were down-regulated in sensitive cultivar Sk101 and not appear in G179 which might contributed to the sensitivity of this cultivar. The whole sequence analysis results are summarized in Table (7).

Further studies may use the identified DD-fragments as probes in order to isolate the corresponding full length genes. These stress genes could ultimately be used in the production of new transgenic plants that will be tolerant to drought stress, and which can be used in land reclamation programs.

SUMMARY

Rice (*Oryza sativa* L.) is a semi-aquatic plant, grow well in tropical, subtropical and temperate regions and it is highly affected by water shortage. Differential display reverse transcription (DDRT) technique was used to investigate differential gene expression between two Egyptian *Oryza sativa* cultivars under drought treatment, in an attempt to identify stress genes whose expression is regulated by drought and define their function. Fifty-six and fourty-seven fragments were differentially expressed under drought in G179 and Sk101, respectively. These differentially display (DD-PCR) fragments were categorized into up- and down-regulated fragments. DNA sequences of 17 fragments out of the total DD-PCR fragments was subjected to a nucleotide and amino acid sequence homology search through BLAST analysis programs from the National Center for Biotechnology and Information (NCBI). Four of the DD-PCR fragments were found to have the same base sequences showed homology with

chaperone proteins *DnaJ15*. Moreover, another three fragments with the same sequence illustrated a homology with retrotransposon protein, putativeTy3-*gypsy* subclass. Narrowing down the number of DD-PCR fragments to 12 fragments. Sequence alignment analysis identified four fragment sequences with significant homology to ribosome biogenesis protein WDR12, transposon protein, putative, CACTA, En/Spm sub-class, cytokinin riboside 5'-monophosphate phosphoribohydrolase LOGL10 and peroxisome biogenesis protein 12 of *Oryza sativa*. Real time PCR for three genes cytokinin riboside 5'-monophosphate phosphoribohydrolase LOGL10, peroxisome biogenesis protein 12 and retrotransposon protein, putativeTy3-*gypsy* subclass were done to confirm their concentration.

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Table (1): List of primer used in DD-PCR.

Primer's names		Primers sequences
Anchored primers	$T_{11}A$	5' TTT TTT TTT TTA 3'
	$T_{11}C$	5' TTT TTT TTT TTC 3'
Arbitrary primers	HAP2	5'- AAG CTT CGA CTG T -3'
	HAP3	5'- AAG CTT TGG TCA G -3'
	HAP4	5'- AAG CTT CTC AAC G -3'
	HAP26	5' TGC CGA AGC TTG CCA TGG 3'
	HAP28	5' TGC CGA AGC TTA CGA TGC 3'
Actin primers	Forward	5' CAG TTC TGA CCA ATC AAA CGA ACC 3'
	Reverse	5' CGC ATG TAT TGG AGA TTT GCA GGC 3'

Table (2): list of real-Time primers for interested genes.

Primer	Sequence		Product size (bp)
Peroxisome biogenesis	F	5' CCTCTGCCAAAGGTTGCTAA 3'	187
	R	5' CTGTGGCAGGCATCAATGTG 3'	
Cytokinin riboside 5'monophosphate	F	5' CGGTGGAGGAAGGGTTCAT 3'	235
	R	5'TCCTCCTCCTACGTCTTCTGA 3'	
Retrotransp-oson protein	F	5' CGCAACGTTGAAGCCTATGT 3'	229
	R	5' TTGGCGGTTTCATGTTGAGG 3'	

Table (3): the expression pattern of DD-fragments.

Cluster	Pattern	Control	Drought	Giza 179	Sakha 101
				DD fragments	DD fragment
Up regulation	1	-	+	SAP26-7, SAP3-3, SCP4-17, SCP28-11, SCP28-12, SCP28-13, SCP28-14, SCP28-15, SCP28-16, SCP28-17, SCP28-18, SCP28-22, SCP28-23	SAP26-3, SAP26-4, SAP26-6, SAP3-9, SAP3-17, SAP3-18, SCP28-3
	2	-	++	SAP2-4, SAP2-5, SAP26-6, SAP3-2, SAP3-8, SAP3-15, SAP3-19, SCP28-19, SCP28-21	SAP2-1, SAP3-19, SCP28-1
	3	-	+++	SAP3-1	
	4	+	++	SAP2-2, SAP2-3, SAP3-4, SAP3-13, SAP3-14, SCP4-2	SAP26-5, SCP4-6, SCP4-7
	5	+	+++	SCP4-1	SAP3-20, SCP4-3
	6	±	+	SAP3-5, SAP3-6, SAP3-11	SAP26-7, SAP3-16, SCP28-7
	7	±	++		SCP4-4, SCP4-18, SCP28-8, SCP28-9, SCP28-10
Down regulation	1	+	-	SAP26-2, SCP4-6, SCP4-16, SCP4-20, SCP4-21, SCP4-22, SCP4-23, SCP4-26, SCP4-27, SCP4-28, SCP4-29	SAP3-7, SAP3-11, SAP3-12, SCP4-9, SCP4-10, SCP4-11, SCP4-12, SCP4-13, SCP4-14, SCP4-15, SCP4-19, SCP28-4, SCP28-5, SCP28-6
	2	++	-	SAP26-1 SAP3-7, SAP3-9, SCP4-5, SCP4-24, SCP4-25, SCP28-20	SAP26-2, SAP3-14, SAP3-15, SCP4-8
	3	+++	-	SAP3-12	SCP28-2
	4	±	-	SAP3-17	SAP3-13, SCP28-11, SCP28-12, SCP28-15
	5	++	+	SAP3-10	SCP4-16
	6	+	±	SCP4-7, SCP4-30	

Table (4): sequences of selected cDNA fragments from the combination of anchored primer $T_{11}A$ and arbitrary primer AP3

SAP3-1 (1198 bp)

AAGCTTTGGTCAGCAGCAGCACATCCACATGACAGCAAGCATGAGGCAGAGCAAA-
GCAGTACAGAGCAGAGGAGGAAATACATATGGAGGGATGGAG-
GAGGCTCATTTCCTCAAGGCGACGGAGTTGTCCATGAAGCCTGCCATCACAC-
CTACAGGTGTAAACGTGTCATCTAATT-
GCTGCTTCGCGGCTTCCTGCTGAACTCCTGAATCCTGTT-
GCTGTGGTGCCGGCGTGCCGCCCTGCTACTGCTGCACTGCTGCTCTATGATTCG-
GAGGACTGGAGTACGTCTGGGGCTCTTGCACTGAC-
GAAATGTACAAACATCCCAGCTTTCATGCTTTCCTTGACTGCCATACTGCCACTT-
GTGCTTCTGCGCTTCTTGTATGCCTGCATACCTGCTCAGCTAC-
CTCCTTCCCTTTCTCGTTCTCATATTCGTAGCTCATTTCCTCAAATCACATT-
GTGCCGCTGAGCTGACTATGACACTCTTACCAGACTGCCTCACAATCAC-
TAATGCACTCGCGACCTCCCGCCCGCCTACCAGACGACAAAGCTCCAAACCTCCTG-
GATCCTTAGATTCATTACTCGATTATGCCATCTTGTCACCTTGGCATCTTCAC-
GTACTCATGTGCATCCCGGGTTCCAGTGTGATCCTCTCATCCTTTCACAC-
TTCCTCCCAACCTGAAACCCAGAATCTTAAACCG-
GAGGTCCCGAATTACTGAACTAACGCGCTTTCTCTGCTTTGCTGCTCAC-
TGCCTCCTTTCCCGTCTTTCAAATCTGTCATGCCGTCTTGCCCTACTG-
CATTCAGCCAATCCGCCGAGGACACGCGGAATAGCCTATTTGGCCTCTT-
GTGCCGCTTCCTCCGCTTCCTGGACTCACTGACCCCCCGGTCGCTTCGGTTGCTGCG-
GATGCGGTATAGGCGTCATTCGAATGGCTG-
TAAATGCCGGTTATTCCCGCTTAATCCACGGGAATCACGGCGAGTAACGCAC-
CGTGTGAACCATGTGGGCCCACAAAGACGGGCCAAAAAGCCCTAGAAAAC-
GGCCCAAAATTGCCCGCCGTTTTTTGCCATTTTTTTCCCTAGGCCCCCTGGAC-
CAACCTTGACGAAAAAATCCCACAGCATTCTAGTCCCCCAAAGTT-
GCCAAAAAATCCCCCAAACACCCCTCAGTAGGAATTTTACAAGAGAT-
TTTCTCCCCCTTTTAACCGC

SAP3-2 (461 bp)

AAGCTTTGGTCAGGTAACAGCAGAAACGCACTGCGTATGTCCCAGAAATGTT-
GAAGTGGCAGAACCCTGAAGTGCAACATTCCAAACTGAGATCAGCAGGGTT-
GTATCATGAGTTCATGACAATAAATAGTATAAAACTTCACAATAAAAGAATT-
GCCAGAATTAAATCTCCCCCAAATTTTAGCAGGTGCATATTAAAAATTATCAAA-
GCACTGGAAATTCAAAATCAATACCCAGCACATTATAAAGCCAAATAACTG-
GAGCCAATTAACCAAGAGATGAACATATTTAGTACTTCACATGATGGCAC-
CATGCACATAAGACCAAAGAATTTGGACTGGAAAGTAAAATGACCTCAAACAGA-
GATTCTTCAGGTCCAGATGAATCAGAATTTGTCCTTCTCTTCTTCACTGAGACAG-
TGTCACCATCTTCTTCCGACCCTTCGACTGACCAAAGCTTA

SAP3-3 (536 bp)

AAGCTTTGGTCAGCAGCAGCACATCCACATGACAGCAAGCATGAGGCAGAGCAAA-
GCAGTACAGAGCAGAGGAGGAAATACATATGGAGGGACGGAG-
GAGGCTTATTTCCTCAAGGCGACGGAGTTGTCCATGAAGCCTGGGA-
TAACACCCTGACCCTTGCATTGAATCTAGGTACCTGCCAGCAAAATTAAAGGTG-
GAAGCTAATCATTTAATTGCTGCTTCTTGGCAAACTCCTGAATCCAA-
TAGCCGTGGTGCTGGTGTGTCACCCTGCTGCAGCCCTATTGATGCACTAC-
CATTCCGTGGTCTGGAAGACCAGTGTGGGTCTTGCACTGGCAAAATGAAGAAA-
TATACCAGCAACAAGGCTTTCATTGAAAGCAAGACTGCCATTTTGTCTCATGTGAT-
TCACCTCAGCTTGGAAGCCTGCAGAGCTAGTCCATAACCTCCATCAATTTCTT-
GTTCGAAGAAAAGTAGCTCATATCTTAAATCTACTTT-

GTGCGGCTGAGGTGACTCTGACTACAATGCCTGACCAAAGCTT

Table (4): Cont''

SAP3-20 (474bp)

AAGCTTTGGTCAGCCTCATAAATATCGCCCCACTGTCATGTGGGGCTACGTCGTAG-
TGGGACCCACCTGAACAGTGTCTCGATGCAGTTAACACACAGGTCGACTCGGACTT-
GCAATTTGCAAACCGGGTCGCAAGATGACGTGTCGACCGTCCATTGG-
CAAATTTTATTTTATTTTTTTCGAATATAAGTCCCTTGGCAAATTTATAA-
GCTTCGTCGAAATCCGAACAGAAAAAATCGGCACCTCTCGATAAAATCTT-
GATTGGTCCTGTAGTTACTGTTCGTCGTGTTTCTACTGTTTCTTTCGTGGACTGA-
TAGGTTTGCTGTGCCGCTCGTGACCTCCCTGATTTGATGCTCTGCTTCTAGAT-
TCTCCTTTAATTAATCGGGGTTTGCGAGCAAGATTAAGTGACTGTTTCTTC-
TATTGTATTGTGTAATTAAGTAGATAATAACCCGCTGGATAATCTGACCAAAGCTT

Table (5): Sequences of selected cDNA fragments from the combination of anchored primer $T_{11}C$ and arbitrary primer AP4.

SCP4-2 (1261 bp)
AAGCTTCTCAACGCAGATCGGCCGCAACGTTGAAGCCTATGTGGATGACGTAG-TCGTCAAGACCAAGCAAAAGGATGATTTGATCTCGGATCTAGAAGAAACCTTT-GCGAGCATCCGTGCTTTCAGGATGAAATTGAACCCTGAAAAGTGCACCCTCGGAG-TACCGTCAGGAAAGCTGCTTGGCTTTATGGTGTCTCATAGGGGCATCCAA-GCCAACCCGGAGAAAGTCACTGCTATCCTCAACATGAAAC-CGCCAAGTACCCAGAAAGATGTTCAGAAGCCGACTG-GATGCATGGCGGCGCTCAGCCGATTTGTTTCAAGACTTGGCGAGCGGGG-GATGCCCTTCTTCAAGCTACTGAAGAAAACAGATAACTTCCAGTGGGGACCCGAA-GCACAGAAGGCCTTCGAAGATTTCAAGAAACTCCTCACCGAGCCACCAG-TCTTAGCCTCACCACGCCCGAAGGAGCCGTTGTTGCTGTATGTATCAGCCAC-CTCTCAGGTTGTGAGCACAGTCTTGGTCGTTGAGAAGCTTAAGCTTCTCAAC-GCAGATCGGCCGCAACGTTGAAGCCTATGTGGATGACGTAGTCGTCAAGACCAA-GCAAAAGGATGATTTGATCTTGGATCTAGAAGAAACCTTCGCGAGCATCCAC-GCTTTCAGGATGAAATTAAACCCTGAAAAATGCACCTTCGGAGTAC-CGTCAGGGAAGCTGCTTGGTTTTATGGTGTCTCATAGGGGCATCCAAGCCAACCCG-GAGAAAGTCACTGTTATCCTCAACATGAAACCGCCAAGTAACCAGAAA-GATGTTCAGAAGCTGACTGGATGCATGACGGCGCTCAGCCGATTTGTTTCAA-GACTTGGCGAGCGGGGGGATGCCCCTTCTTTAAGC-TACTGAAAAAAAAACAAATGAATTTCCAGTGGGGGACCCGAAA-GCACAGAAAGGCCTTCGAAAAATTTCAAGAAAACTCCCTCAACCGAGGCCAC-CGGGTCTTAACCCTTCACCACACCCCGTAAGGAGCCCGTTGGTGGCTGG-TATGGTTTCACCCCCCCTTCCCCAGGTTTGTGGAGGTACAAGTCTTTGGGTGGTTT-GAGAAAAACTTAAATCCCCCACTGGAAATTTCGCGGGCCGCCCCGGGCCGGGTCG-CACCCATATTGGGGGAGAAAACCCCCCCCACCCCCGTTGGGGAATGGCAAAAAC-CTTGGAGGAAATTTCTATAATGGGTCGCCCCAAAAAAAAACCTTT-GGGGGGTGAAAACCAGGGGGC
SCP4-3 (829 bp)
AAGCTTCTCAACGTCGACGGGTACTACAACTCGCTGCTGAC-GTTCATCGACAAGGCGGTGGAGGAAGGGTTCATCAACACCAGCGCGCGGCG-GATCATCGTCATGGCGCCGACGGCGGAGGAGCTGATGGACAAGCTAGAGGTACAC-GATGACTGACAAGACAGTCACTCGATATATCGAGCTT-GGGTTTCAGACTTTCAGGTTCTTGCATTGCAGACCTCTGAACATT-GCAGACTCTGATGAACATTTCTGCTGCAGGAGTACGTGCCTTACCAC-GACAGGGTGGCATCGAAGCTGAACTGGGAGATGGGCCACCTAGGTTACTGAC-GTACTCACTTACAGCTGCAGCAAATTGCCAGACAG-CAATGGCGATCGATCGATCAGAAGACGTAGGAGGAGGAGGAAGAAGAAGAGAA-GAATCAAGTCGATCGACTCATCATCAAGAAGCTGGGAAAGATTTGGTT-GATGCAGCTAGAAAGCCAAGAACGAGAGTGTGGCGTGCGTGCGTCCATTTT-GTATATGTTTGCTTCGATCAGGGGCAGCTTGAATACTGTAGTAACTTGGATGCTT-GATGTCCTCTTGATCTTCTACCTAGCTACCGAGATAAGAACTAGTAGTAGTAG-TTAGTAGTAGGTAGTAGTAGTAGTAAGTAGTAGTTAGTATTCCAGTAGCGTATGG-TATGTCAGTTAATTTTCAAGGTTATCCAGAATTCCGATTGAACCACAGCCAAAGTT-GTTTTCTGTAATCACCCCTCGTTAAAGGCAATGGTAGTGTTAAGCTAAAACGTTT-GAAGGAGGCTTGAAGGCGTTCGTGTGATGAGGAGCACGA

Table (5): Cont''

SCP4-4 (672 bp) AGTGGGTCCCCATCTGACGGCTCGAGCCCTGGCGGCCGCGCGGCAGCTGTTATTC-TATTAGCTCTCCTCGGCCGATACATTACCTGGAGGGCAGTGTGGCTGCGCG-TAATGAGACCAACTTGATTAGAGTCGGAAGCTGTCATGGTACAGGTCCAAGGGA-GACTGATTTTCATACCGCGTCGAAAAAAAGTTTCGACAGTCCATTGCGGAT-TTTATTTGATTGTTGCGAATAATGGGGTTGCCTGTTTCCTGGTTTGAC-GAAATGAAACCACAAAATGTCAGGCCTCTCGAAAAAAATTGAATGGTAC-GGAATGGATGTTCACCCAGTTTCGCTGGATCTCTCG-GAAATGACAGGTCTGGTGTGCCGCTTTTGAGCTCCCTGA-GATTAGCTGATCTTCAATGGGGACTTCACTGACCTCGGCTTTGGAAACCAATTAG-TGAGTGTCTAGTCTATTAAATAGGTCGTTGGCAAAAAATAATCTGGTGGATAAC-CGAACAAAAACATGGTGGGGGGGGAATTTGCCGAGTTGTAAGTCCTTATGTGAA-TAAATTCTAAGCTTCTTGGGTGGATTCTTGTTGATA-CATCCCCCCATATTAAGTGTTTTTAGTTGGTAGTATGGAATCAGGGGGA-GATGCGTGTCCTGGCATCTCCAAAGAGAAATGCCCCATCCA
SCP4-6 (677 bp) AAGCTTCTCAACGTGGACGAATGGAAGTGGATGCTTCACATCAA-GAACTTAATGTGGGAAGGTCTGCAGGAGCACCGTATTTATCCGCCTGAGTCG-GAGGCACTTGGCCGGAAATTTGCATTGCAAACAATT-GCCCATCGGTGGCGACAATGGAAGTCGGACATGAACACCAATTATGTTCAGAA-GAATAAGACGCCATTTGAAGAATGGGGCACCAATTCAGCATTGAGTGGGG-CAAGTTCGTGGTTCCATGATTAACCTTCTTGCACTGAGGGGATGAGGAAGATTT-GCAGAGGTTTGGAAGGGCCTTGTTGTGTCGCATTGACCCATAAGTTATTAAGTAA-GCGACGAAAGAGTGAAATCTAATCAGCAATGGGGACCTGTACAACAAAA-GAGGCGCGATAGCTCAAAATACACGAAAACGAACCAATTGATTATAGAACTGAGC-TACAAGGAGCGATAGTGGCTGTAATTGGAAAAAAAAACATGAGGTGACCTGAGA-CATTGTGTGAAAAAAGAAAATAATTTTTGAATTCGTAACCTTTTAATGATCACCA-TAAAGGAGAGGAGCTTCGCAAAATAACATCTCTAAAGTGTCG-CAAAAAAGTCTAAATAGCTAGAGCAAGAAGGGAAGGCGGCTCGTAGTTGAA-TAGGCCATGGGAACGGAGACAGGTTCC
SCP4-8 (588 bp) AAGCTTCTCAACGAGCAGCAAGATGCAACTTATGTTGCTAAC-CTCCAGATGGAGCTCCAGCAAGCACGTGATCGGGTAAGTGAACTGGAAAC-CGAGCGACGTGCGGCTAAGAAGAAGCTGGATCACTTGTTCAAGAAACTTGCAGAG-GAAAAGGCAGCTTGGCGGAGTAGAGAGCATGAGAAAGTGCGCGCAATTCTTGAA-GATATGAAGGCAGATCTTGACCATGAGAAGAAAAACAGGAGACGCCTGGA-GATGATTAACTTGAAGCTTGTCAATGAGTTGAAGGAAGCCAAGATGTCAGCAAA-GCAGCTACTGCAAGAATATGATAATGAGAGGAAAGCACGCGAGCTCACTGAG-GAGGTGTGCAATGAACTGGCAAGGGAGGTGGAGGAAGACAAAGCCGAGATTGAA-GCCTTGAAACATGATTCCCTCAAGCTGCGAGAGGAGGTTGATGAGGAGCGCAA-GATGCTACAGATGGCTGAGGTTTGGCGTGAAGAACGGGTGCAGATGAAGCTTGTT-GATGCAAAACTTACTCTTGATGCCAAATGCACACAATTGAGCAAACTGCAACAG-GACGTTGAGAAGCTT

Table (6): sequences of selected cDNA fragments from the combinations of ($T_{11}A$/AP2, $T_{11}A$/AP26, $T_{11}C$/AP28), respectively

<u>SAP 2-1 (556 bp)</u> CGGCCGCCATGGCGGCCGCGGGAATTCGATTAAGCTTCGACTGTGG- CAGGCATCAATGTGATGGGGCACCTTTTATGCTGTAGAGCAAAACAAAATCAAATT- AGCGACTGATTGGTGGCTAAGATAAATAGATTGTGGGTT- GCTTCTTTCTGATCATAATAATGGAAAGCTTCTTTGTGACAGTAATCTACCTGGGA- GACTGACTTAAAAATGCAACTGTAGCAGAATACAAAACCAGAAGCAGAAA- GAACAGAAGGGTTATTGCGCTTCTGGCAGCATAAGGGG- CACAATGTCCTGTCAGGGGGCAAGGGAAGTCCATCTTTAGCAACCTGATGAAA- GAAGTAGACGCTCAAATCAAACAGTAGTCATATAAAGAACTATTAAAC- CACAAAATATATCCAACTGCACAGTGGCAAAACAAAGAGTTCTGTTCAC- CATGTCACTGCAAATTGAGATTTCAGACAAGGGTAACTGCTTTCTGCATA- TATTGACTAGCATATAACATGTACCTTTGGCAGAGGTGGAGGAGGGGGTGGGGGA- TATACAGTCGAAGCTT
<u>SAP26-6 (640 bp)</u> TGCCGAAGCTTGCCATGGTGATACACTTGAGGTAGAACGGAGAGGCAAGAACGTG- CACTGATGGTTCCGATAGACGG- TACAACTCCCGATGCCTCCCATACCTACAACTTCGAATGGAACATATCAG- GATCGTCTGTCCTTCTCCATCAGCTTTCGAGC- TACTCTCATCTCTTTTATGTGCCAAAATATATTAGATTGTTTATA- TATCATCAAAATCAAATGTATTTTTGACGCGTGCAGCGGTGTTCAAATGGGCAC- TGAATGCAGAAGACATGGTAACCTATAATCTTGACGAC- CATGCCTTCTGATGACTTAATTTTGAGTAATCCCCAAACGGGTGGAGTCATGGAGA- GATGAGATGAGATCGACTTAACTACCTGATGATACTGAACAATTCATTACTG- CACAAAAGAGTGTTCAGTATCTAAATATCTAATTTCCCTGCAACAATTCATAAC- CTTCAAATCTACATTTCAGTTAGAATTGCATCTGATTCG- CAGCCTCAGATCAGATGCGCGGTGCCATCACATAACAGAGCAAGATCAC- GTCATCACAGTCACAACGGTCTTTGAACCTGCACCACTACAGTAGTG- CAGAATGCACTGCTTGCCATGGAAAGCTTCGGCGAATCACTAATTGA
<u>SCP28-2 (324 bp)</u> TGCCGAAGCTTACGATGCCCAAAGAAAAAATAAACAAAGTTCGATTCAAAATAA- GCTAAAAATTAAACCAACGGACTCTGAATCAAACTCAATAGAATTAGCTGGGAG- GATCCACATGATTAAAAGCTGGAAACCAACGGAATCGAGCAAATCACAAAGCAA- TACGTCATATCTGACCGGCGACAAGCTCTCGTCCGACAAGAGGATTGGCGGTT- GTGGCGGACGAAGATGGAGACGAGAGGTGAGCTCCAACGGCTCAAGACAC- GCCGTCGCTGAGGGTGCTCCGCCGACCCGTAGGACACTGCTGCATCGTAA- GCTTCGGCA

Table (7): Summary of the sequence analysis.

Fragment ID	Molecular size	Homology results Genbank Accession number, E-value	Blast X
SAP3-1 (179t)	**1198 bp**	**chromosome 1, BAC lone:OJ1111_G12 (OsJ) Seq. ID: AP003337.2 E: 3e-62 I: 97% Score 251 bits +/- (Gojobori *et al.,* 2002)**	**hypothetical protein OsJ_01630 Seq. ID: EAZ11762.1; E: 2e-06, I: 38% Score 57.8 bits (+2)**
SAP3-2 (179t)	**461 bp**	**chromosome 7, BAC clone:OJ1634_B10 (OsJ) Seq. ID: AP003840. E: 0 I: 99% Score 810 bits +/+ (Yamamoto *et al.*, 2001) ribosome biogenesis protein WDR12 (LOC4343819), (OsJ) Seq. ID: XM_015792429.1 E:1e-43 I: 99% Score 188 bits +/-**	**ribosome biogenesis protein WDR12 [OsJ] Seq. ID:XP_015647915.1 E: 3e-11, I: 100% Score 70.5 bits (-2)**
SAP3-3 (179t)	**536 bp**	**Chromosome 1, BAC clone:OJ1111_G12 (OsJ) Seq. ID: AP003337.2 E : 9e-121 I: 9% Score 444 bits +/- (Gojobori *et al.,* 2002)**	**Chaperone protein dnaJ 15 (OsJ) Seq. ID: XP_015622174.1 E: 2e-04 I: 80% Score 38.5 bits (-2) Putative ARG1 protein (OsJ) Seq. ID: AAP06849.1 E: 5e-04 I: 88% Score 47.8 bits (-1) (FAO *et al.,* 2002)**
SAP3-20 (101t)	**474 bp**	**Chromosome 9, BAC clone:OJ1740_D06 (OsJ) Seq. ID: AP005579.3 E:0 I: 99% Score: 834 bits +/- (Katayose *et al.*, 2002)**	
SCP4-2 (101t)	**1261 bp**	**Chromosome 6 sequence (OsI) Seq. ID: CP018162.1 E: 0 I: 98% Score: 910 bits +/- (Liang *et al.,* 2017)**	**Retrotransposon protein, putative, Ty3-gypsy sub-class, expressed (OsJ) Seq. ID: ABA97388.2 E: 2e-98 I: 90% Score 315 bits (+1)**

Fragment ID	Molecular size	Homology results Genbank Accession number, E-value	Blast X
SCP4-3 (101t)	**829 bp**	**Probable cytokinin riboside 5'-monophosphate phosphoribohydrolase LOGL10 (LOC4348914), mRNA (OsJ) Seq. ID: XM_015757848.1** **E:0 I: 94% Score:778 bits +/+**	**Probable cytokinin riboside 5'-monophosphate phosphoribohydrolase LOGL10 (OsJ)** **Seq. ID: XP_015613334.1** **E: 1e-32 I: 64% Score 128 bits (+1)**
SCP4-4		**NS**	**NS**
SCP4-6 (101t)	**677 bp**	**Chromosome 7, BAC clone:OSJNBa0077F02 (OsJ) Seq. ID: AP005247.3 E: 3e-103 I: 91% Score: 387 bits +/-** **(Katayose *et al.*, 2002)**	**transposon protein, putative, CACTA, En/Spm sub-class (OsJ) Seq. ID: ABA98560.1 E: 2e-30 I:81% Score 128 bits (+1) (Katayose *et al.*, 2002)**
			hydroxyproline-rich glycoprotein -like [*Oryza alta*] Seq. ID: BAX25052.1 **E: 4e-25 I: 73% Score 113 bits (+1) (Gojobori, *et al*., 2002)**
SCP4-8 (101c)	**588 bp**	**Chromosome 4, BAC clone: SJNBa0053K19, *(Oryza sativa*) Seq. ID: AL606645.2 E: 0 I: 99% Score: 1036 bits +/-**	**Hypothetical protein OsJ_16230** **Seq. ID: EAZ32049.1** **E: 4e-124 I: 98% Score: 376 bits (+1)**
SAP2-1 (101t)	**556 bp**	**Chromosome 10 BAC OSJNBa0006L06 *(Oryza sativa*) Seq. ID: AC022457.8 E: 0 I: 99% Score: 924 bits +/- (Fraser *et al.,* 2016)**	**Putative peroxin (OsJ) Seq. ID: AAK27808.1 E: 2e-26 I: 61% Score 112 bits (-3) (Fraser *et al.*, 2016)**
		Peroxisome biogenesis protein 12 (LOC4348849), (OsJ) Seq. ID: XM_015757410.1 E: 3e-63 I: 100% Score:253 bits +/-	**Peroxisome biogenesis protein 12 (OsJ)** **Seq. ID: XP_015612896.1** **E: 2e-26 I: 61% Score 113 bits (-3)**
SAP26-6 (179t)	**640 bp**	**Chromosome 12. BAC OSJNBa0010M16 of library OSJNBa Seq. ID: AL831797.4 E: 0 I: 99% Score: 1070 bits +/-**	

Fragment ID	**Molecular size**	**Homology results Genbank Accession number, E-value**	**Blast X**
SCP28-2 (101c)	**324 bp**	**Chromosome 12, BAC OSJNBa0036H15 of library OSJNBa Seq. ID: AL844879.5 E: 7e-152 I: (99%) Score: 547 bits +/-**	**Hypothetical protein (OsJ) Seq. ID: BAC83600.1 E: .036 I:79% Score: 40.4 bits (+2) (Yamamoto *et al*., 2001)**

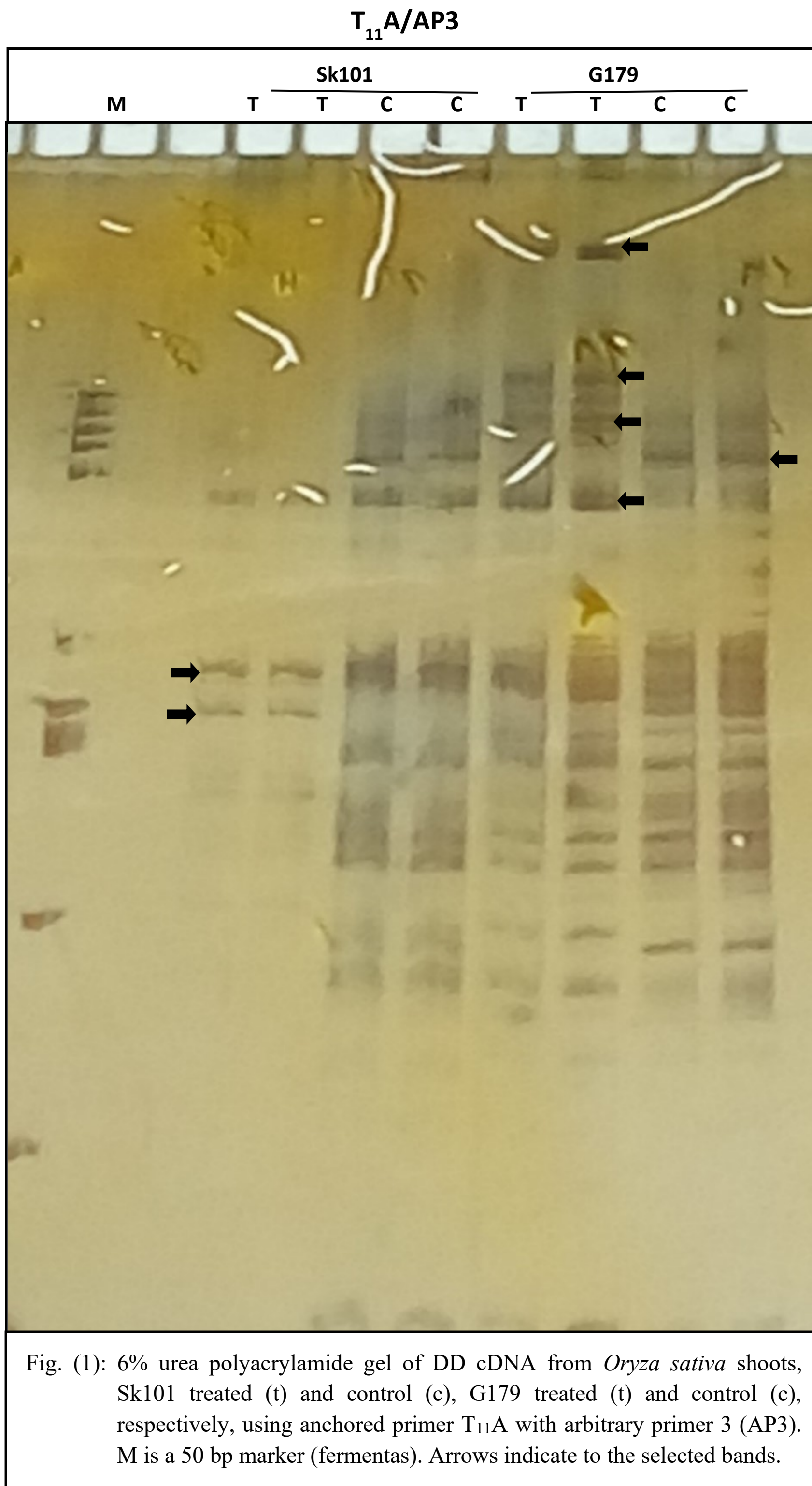

Fig. (1): 6% urea polyacrylamide gel of DD cDNA from *Oryza sativa* shoots, Sk101 treated (t) and control (c), G179 treated (t) and control (c), respectively, using anchored primer $T_{11}A$ with arbitrary primer 3 (AP3). M is a 50 bp marker (fermentas). Arrows indicate to the selected bands.

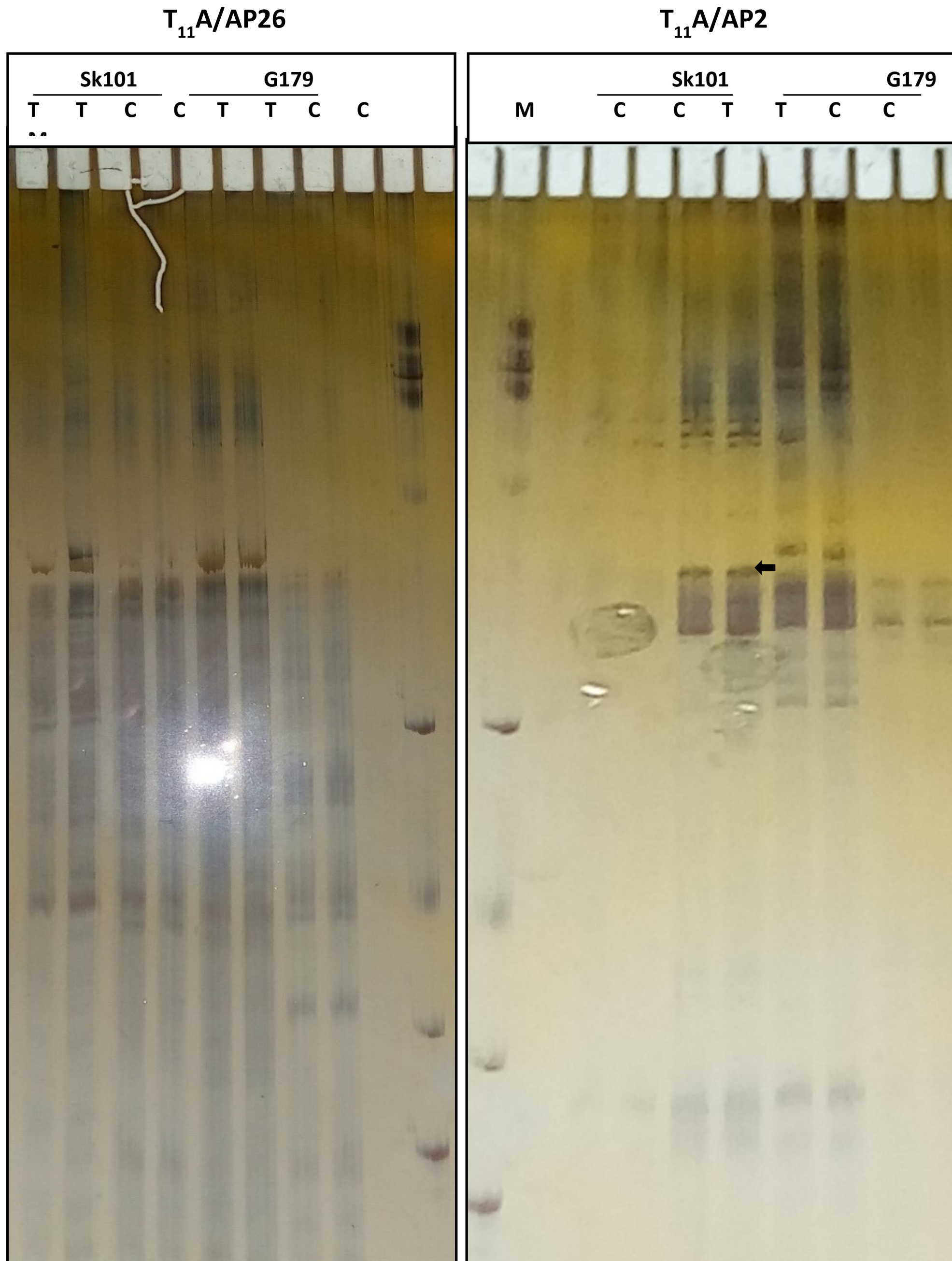

Fig. (2): 6% urea polyacrylamide gel of DD cDNA from *Oryza sativa* shoots, SK101 treated (t) and control (c), G179 treated and control (c), respectively, using different primer combinations of anchored primer $T_{11}A$ with arbitrary primers 26 and 2 (AP26 and AP2). M is a 50 bp marker (fermentas). Arrows indicate to the selected.

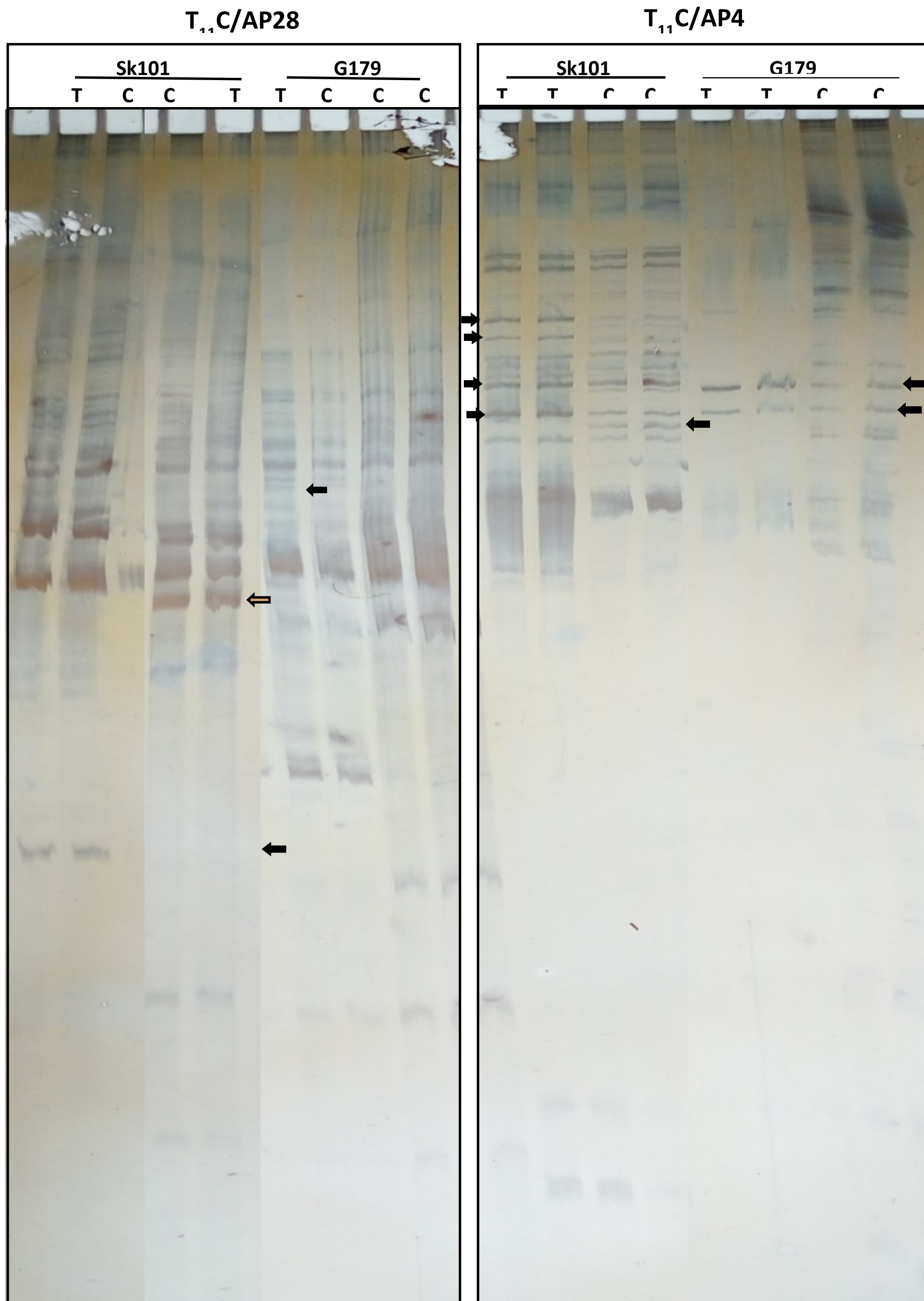

Fig.(3): 6% urea polyacrylamide gel of DD cDNA from *Oryza sativa* shoots, Sk101 treated (t) and control (c), G179 treated (t) and control (c), respectively, using different primer combinations of anchored primer $T_{11}C$ with arbitrary primer 28 and 4 (AP28 and AP4). Arrows indicate to the selected bands.

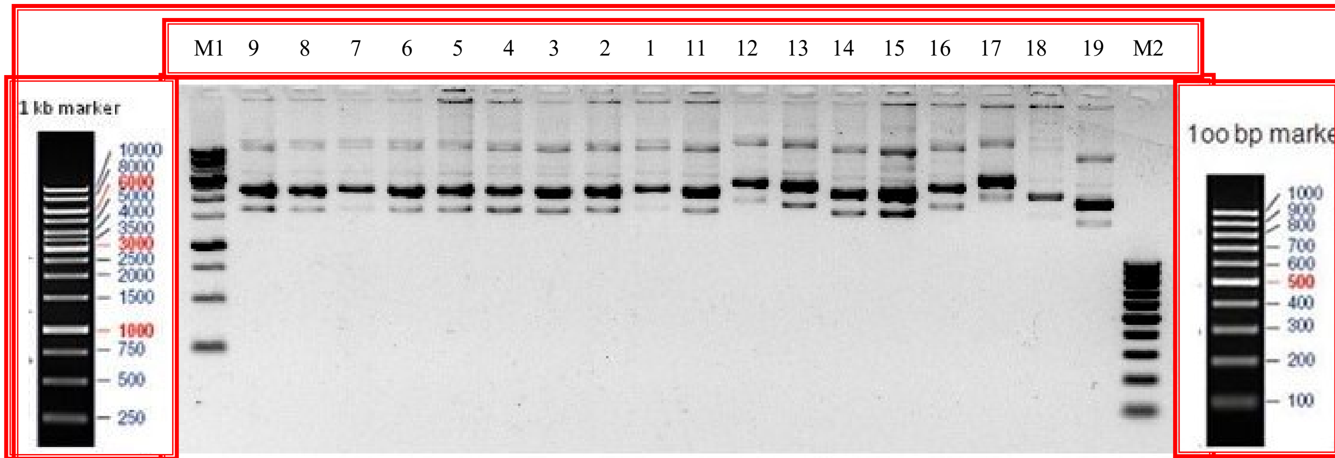

Fig. (4): Analysis of DD fragments. The reamplified fragments were separated on 1.5% agarose gel. Where Lane 1 represents SCP28-1. Lanes 2 to 8 represent SCP4-1 to SCP4-7. Lane 9 is SCP-ve control. Lane 10 is 1 kb marker (fermentas). Lane 11 primers represents SAP26-1; lane 12 represents SAP2-1; lanes 13 to represent SAP3-1 to SAP3-6; lane 19 is SAP-ve control and lane 20 is 100 bp marker (fermentas).

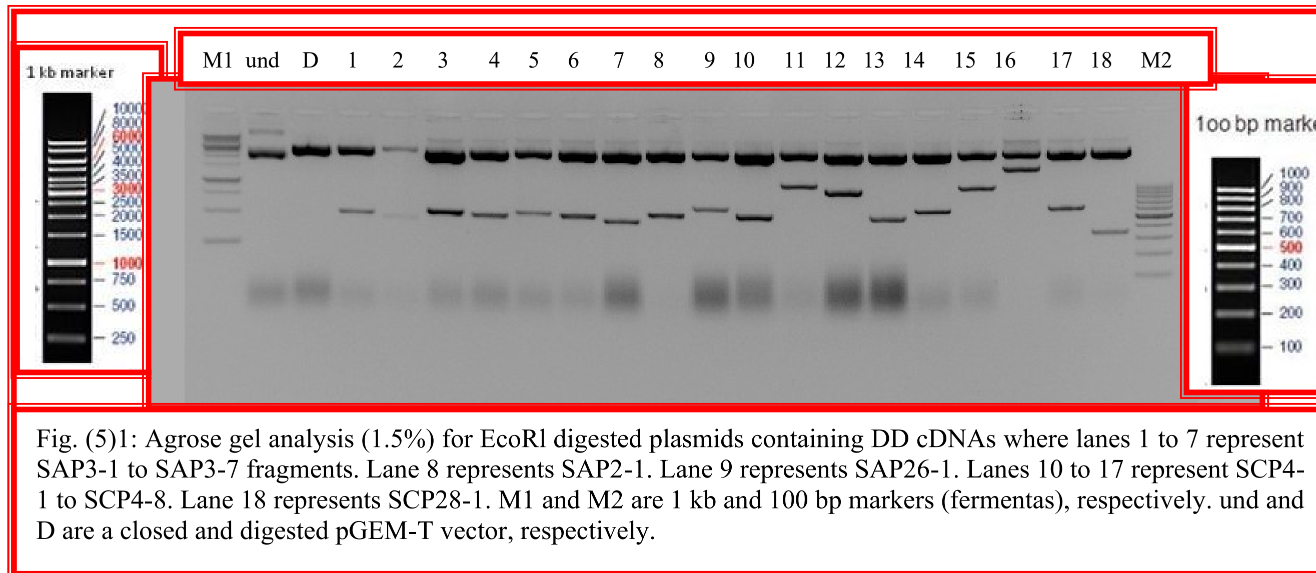

Fig. (5)1: Agrose gel analysis (1.5%) for EcoRl digested plasmids containing DD cDNAs where lanes 1 to 7 represent SAP3-1 to SAP3-7 fragments. Lane 8 represents SAP2-1. Lane 9 represents SAP26-1. Lanes 10 to 17 represent SCP4-1 to SCP4-8. Lane 18 represents SCP28-1. M1 and M2 are 1 kb and 100 bp markers (fermentas), respectively. und and D are a closed and digested pGEM-T vector, respectively.

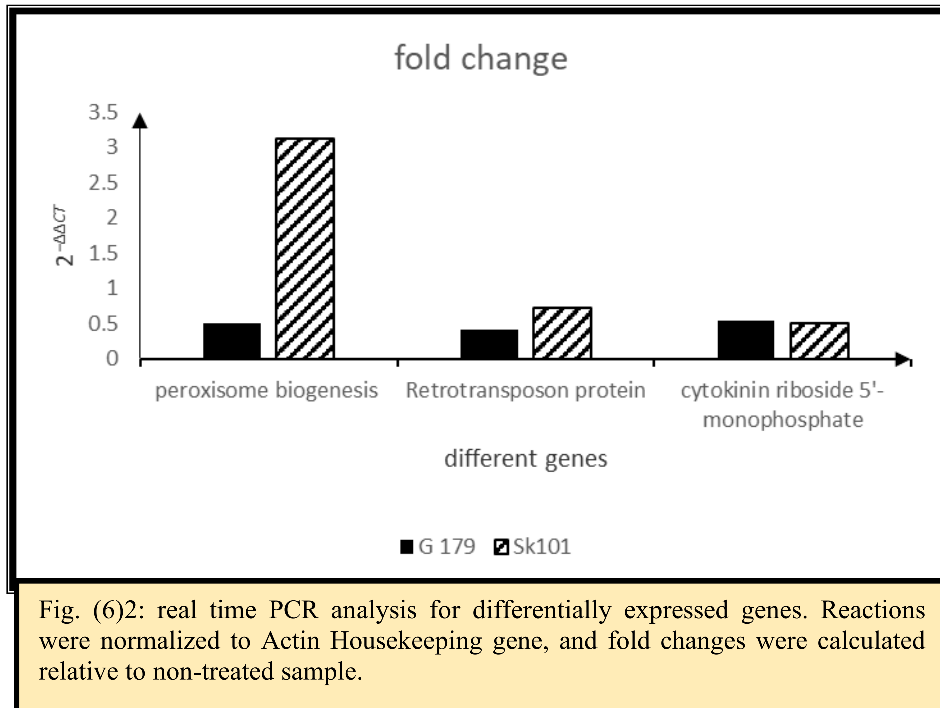

Fig. (6)2: real time PCR analysis for differentially expressed genes. Reactions were normalized to Actin Housekeeping gene, and fold changes were calculated relative to non-treated sample.