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Changes in Protein Expression in Peanut Leaves in the Response to Progressive Water Stress

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ABSTRACT

This investigation was carried out at the Department of Plant Science and Agricultural Resources, Khon Kaen University in the rainy season of 2011. The objective of this study was to identify proteins in leaves of drought-susceptible peanut plants when regulated by progressive water stresses. The drought-susceptible peanut plants of Khon Kaen 4 cultivar were grown in pots under controlled environment. At day 30 after seed emergence, the plants were subjected to stress conditions for 5 and 6 days. The results showed that withheld water supply for 5 and 6 days gave moderately and severely water stresses, respectively. Under moderate water stress conditions, two up-regulated and eight down-regulated proteins were attained. The up-regulated proteins were striated fibre assembling and flap endonuclease 1. The down-regulated proteins were peptidyl-prolyl cis-trans isomerase FKBP4, tRNA(Ile)-lysidine synthase, chloroplastic, chloroplastic thioredoxin F-type, cytidinedeaminase 7, ALF domain class transcription factor, nudix hydrolase 8, pentatricopeptide repeat super-family protein, putative and ribulose-1,5-bisphosphate carboxylase/oxygenase, a large sub-unit. Under severe water stress conditions, two proteins, i.e., tRNA(Ile) lysidine synthase, chloroplastic and ALF domain class transcription factor did not change their relative abundance significantly where it indicated drought acclimation. The remaining proteins displayed significant changes and the changes were the same as those found in the peanut leaves when deprived water for 5 days. Up-regulated proteins are responsible for alleviating oxidative damages to plant genome and mediating plants responsive to the environmental factors in providing mechanical support, barriers and a rapid transport route. Down-regulated proteins were associated with drought susceptibility of the Khon Kaen 4 peanut plants.

Key words: Peanut, water stress, 2-DE, plant proteome, mass spectrometry

INTRODUCTION

Peanut or groundnut (*Arachis hypogaea*) is one of the most important leguminous crops where matured seeds in pods provide edible oil and protein for human consumption. It is widely grown in the tropics and sub-tropics mainly under rain-fed conditions where drought conditions frequently occur. It has been advocated that when stress conditions occur at a critical growth stage it may result in a drastic reduction or even a failure in crop production (Wright and Nageswara Rao, 1994). Most crop plants possess some mechanisms to avoid drought stress conditions by maximizing water uptake as well

as minimizing water loss. Maximization of water uptake occurs when more assimilates are invested in root proliferation at its depths where water is available. Water loss could be minimized by several mechanisms e.g., a partial closure of stomata in reducingthe absorption of light energy through the rolling leaves and the increasing reflectance ofa dense layer of trichomes, and the reduction in leaf areas, and a rapid senescence of the older leaves (Lopes *et al*., 2011; Marshall *et al*., 2012). Stomata limitation took place during water deficit stress condition could be effectively resulted in a reduction of photosynthetic CO_2 assimilation. As a consequence, the requirement for ATP and NADPH in photosynthetic carbon reduction cycle or Calvin Cycle declines considerably. Light absorption exceeds the capacity for its utilization in the carbon assimilatory reactions and excessive light energy will eventually lead to the production of Reactive Oxygen Species (ROS). The ROS includes superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^{\bullet}$), and singlet oxygen ($^1O_2$) as reported by Asada (2006). The ROS accumulation during stress condition greatly depends on the balance between the ROS production and the ROS scavenging. Production and the removal of the ROS must be tightly regulated. However, the equilibrium between production and scavenging of the ROS may be perturbed by a number of the adverse environment stresses including drought conditions. When the level of the ROS exceeded the defense mechanisms, a cell is said to be in a state of oxidative stress. The ROS induces a broad range of DNA damage, including base and nucleotide modifications, especially in the sequences under the high guanosine content, and may even cause strand breaks. It is well documented that oxygenated nucleotides like 8-oxo-guanine can cause the accumulation of hydroxyl radicals ($HO^{\bullet}$). The 7,8-dihydro-8-oxoguanine (GO) is a common oxidative DNA lesion generated by a direct modification via the reactive oxygen species. The GO lesions are and can mispair with adenine during the DNA replication (Tuteja *et al*., 2001). The ROS may cause modification of proteins and some are direct and others are indirect. The direct modification involves alteration of a protein's activity through nitrosylation, carbonylationdisulphide bond formation and glutathionylation. Proteins can be modified indirectly by the conjugation with its breakdown products of fatty acid peroxidation. As a consequence of an excessive amount of the ROS production, it establishes hessite-specific amino acid modification, fragmentation of the peptide chain, aggregation of cross-linked reaction products and alteration of electric charge and an increase in susceptibility of proteins to proteolysis (Sharma *et al*., 2012). It revealed that maintaining proteins in their native conformation, it could prevent the aggregation of non-native proteins, refolding of denatured proteins to their functional conformation and the removal of damaged proteins and polypeptides are all critical for cell survival under water deficit stress conditions (Vierstra, 1993).

As sessile organisms, the plants must cope up with their ever changing environmental conditions in order to survive, the plants must have evolved upon its elaborate mechanisms to perceive with a rapidly respond to a diverse range of biotic stresses. Sensing of the environmental stresses may occur after its stress perception. The plants commonly perceive the stresses by plasma membrane located within the receptors, intracellular or cytoskeleton-associated proteins. The stress perception is transmitted using the signaling cascades taken into the altered gene expression programmes and ultimately resulting in both metabolic adjustment and the altered physiological responses (Osakabe *et al*., 2013). Under oxidative stress, the plants may have its strategies to minimize the ROS generation or maximize detoxification of the overwhelm production of the ROS. A strict control of the ROS levels is very important in preventing their toxicity and to ensure an accurate operation of their signaling functions. In chloroplasts, copper/zinc superoxide (Cu/ZnSOD) causes the dis-mutation of the superoxide radicals into hydrogen peroxide and the ascorbate peroxidase (APX) catalizes the conversion of the H_2O_2 to water. In this process, ascorbate (AsA) is oxidized to mono dehydroascorbate radical (MDA), which is regenerated to the AsA by either reducing the ferredoxin or by NAD (pH), which catalysed by an MDA reductase (MDAR). Dehydroascorbate (DHA) is produced when the MDAR was not able to reduce the MDA to AsA. The DHA is then reduced to the AsA by the DHA reductase (DHAR) using a reduced GSH as an electron donor. Peroxiredoxins(PrxRs) together with the Thioredoxin (Trx) can also provide antioxidative protection enabling the detoxification of the photochemically produced H_2O_2 in the chloroplasts (Miller *et al*., 2008). The PrxRs and trxplayits possessed important roles during the drought and oxidative stress conditions. The SODs are the only plant enzymes capable of scavenging $O_2^{\bullet -}$, whereas the H_2O_2 can be catabolized directly by catalases (CATs) or by Ascorbate Peroxidases (APX), peroxiredoxins, Glutathione Peroxidases (GPX) and the heterogenous group of the guaiacol peroxidases (Gechev and Hille, 2005).

Proteins often mis-fold under the stressful environments and the mis-folded proteins are highly deleterious to the cell because they can form non-physiological interactions with other proteins (Chen *et al*., 2010). Repair proteins called chaperones can, in many instances, restore the native conformation of the wrongly folded proteins. Molecular chaperones are responsible for protein folding, assembly translocation and degradation in a broad array of normal cellular processes.They also function in the stabilization of proteins and membranes, and can assist in protein refolding under stress conditions (Wang *et al*., 2004). The changes in a relative expression of proteins occurred under water stress (Riccardi *et al*., 1998) and the changes are highly variable depending on genotypes of the crop plants. The up or down regulated proteins are associated with drought sensitive or drought tolerant varieties of the crop plants (Benesova *et al*., 2012). However, the changes in abundance of the proteins under different magnitudes of water deficit are not well

documented. The objective of this study was to identify proteins being regulated by the different levels of water stress and relate them to the susceptibility of peanut variety of Khon Kaen 4 to drought stress conditions.

MATERIALS AND METHODS

A drought susceptible variety of peanut crop viz., Khon Kaen 4 cultivar was grown in the composted pots and kept in a Climate Simulator (Model 630, Contherm Scientific Ltd., New Zealand) under controlled environments. On day 30 after seed emergence, water stress was imposed to the plants by withholding water for 5 and 6 days, whilst the plants of the control treatment were adequately supplied with the water. For this experiment, water status of soils and plants was determined. Fully expanded leaflets of the plants in the experiment under both water stress and adequate water supply conditions were harvested. They were frozen in liquid nitrogen and kept at -80°C for a few days and then extraction and separation of proteins were taken place. The leaflets of the plants supplied with adequate water and under water stress for 5 and 6 days were used for protein extraction and separation using two-dimensional gel electrophoresis with the method described by Akkasaeng *et al.* (2007). The proteins were separated in three replicated gels and the proteins in twelve gels were resolved. The proteins were visualized by the silver nitrate staining with the use of the methods described by Heukeshoven and Dernick (1985).

Image analysis: Analyses of gels were performed using ImageMaster 2D Platinum 7.0 (GE Healthcare). Two reference spots with iso-electric point (pI), molecular weight (M_r) of 5.21, 40 and 5.78, 43 were assigned for pI and M_r of all the remaining proteins in each gel were estimated by ImageMaster 2D Platinum 7. Volume parameter was used to indicate the relative abundance of the proteins. Protein spots in six gels were matched and the volume means of the protein spots under water-stress and non-stress conditions were compared. The up-regulated proteins were indicated when the volume means were greater when under stress condition whereas the down-regulated was assigned when their volume means were reduced under stress conditions. Three-dimensional views of the proteins were visualized to provide the supporting evidence for the up or down-regulated proteins. For mass spectrometry analysis, an additional of 2-D gels was provided and the proteins contents were stained with the use of the Coomassie Brilliant Blue G-250 (GelCode™ blue stain reagent, Pierce Biotechnology, USA).

Protein digestion and MALDI-TOF analysis

Chemicals: Acetonitrile (ACN) of LC/MS grade was taken from the J.T. Baker chemicals. A chemical of α-cyano-4-hydroxy-cinnamic acid (HCCA) was taken from the Bruker Daltonik GmbH. Whilst the Trifluor acetic acid peptide (TFA), a sequencing grade was obtained from the Applied Biosystems.

Protein digestion: Proteins spots regulated by water stress were excised from the gels. Proteins were digested in the gel plugs following the methods described by Shevchenko *et al.* (2007). Briefly, proteins in the gel plugs were reduced with a 10 mM DTT in a 100 mM ammonium bicarbonate for 1 h at 56°C and alkylated with a 100 mM iodoacetamine in a 100 mM ammonium bicarbonate for 1 h in the dark at room temperature. The protein in each excised gel was rehydrated in a 20 μL of a 12.5 μg mL^{-1} trypsin in a 100 mM ammonium bicarbonate for 20 min in an ice bath. The in-gel tryptic digestion was carried out at 37°C overnight. The resulting peptides were extracted three times with a 50 μL 0.1% trifluoroacetic acid and a 50% acetonitrile for 20 min each. The pooled extracted samples were then evaporated to its dryness. The dried peptides were dissolved in a 3 μL of 0.1% trifluoroacetic acid.

MALDI TOF analysis: For the preparation of matrix solution, HCCA was saturated in the TA (ACN:0.1% TFA, 1:2). Tryptic peptide sample was mixed with an equal volume of a matrix solution and deposited onto an AnchorChip target plate (Bruker-Daltonics). Cocrystallization of the matrix and sample occurred at ambient temperature. The mass spectra were recorded using a reflector viz., Bruker reflex V (Bruker Daltonik GmbH), a delayed extraction the MALDI-TOF mass spectrometer was equipped with a 2 GHz LeCroy digitizer and a 337 nm N_2laser. The acceleration voltage was 25 kV for the IS1 and 20.2 kV for the IS2. A total of 100 laser shots from three to five different positions were summed up for each peptide sample. The peptide data were acquired using the Flex Analysis software (Bruker Daltonics).

Data bases searching: The peptide data was searched and formulated using the MASCOT software (www.matrixscience.com). Two data bases, i.e., Swiss Prot and NCBInr were used. For the MASCOT search value, a peptide tolerance was ±1.2 to 1.8 Da allowing a maximum of 1 missed cleavage by trypsin. Fixed modification and variable modification were carbamidomethylation of cysteine and an oxidation of methionine, respectively. Identified proteins had its MASCOT scores with probability $p<0.05$.

RESULTS

Withholding water supply for 5 and 6 days severely affected Leaf Water Potential (LWP) as compared with the controlled treatment. The LWPs for the non-stressed and stressed plants under the withholding water supply for 5 days were -0.20 and -0.42 MPa, respectively. On day 6 of the withholding water supply, the LWPs in the non-stressed and stressed plants were -0.23 and -1.11 MPa, respectively (Table 1). On day 5 of the withholding water supply, there were some significant differences in Relative Water Contents (RWC) between the non-stressed and the stressed plants. After the withholding water supply for 5 days, the percentages of the RWCs were 93.7 and 69.4%, respectively. With the

withholding water supply for 6 days, the result showed that the RWC values for the non-stressed and stressed plants were 94.1 and 61.1%, respectively (Table 1).

Reproducibility of the gels and regulated proteins by water stress: Protein spots were clearly resolved and proteomic maps were very similar across the 6 gels using the proteins from leaves of the plants with adequate water supply and water stress for 5 and 6 days (Fig. 1). The total of 1,092 and 1,600 protein spots were reproducibly detected in leaf proteomes of the peanut plants with adequate water supply and those subjected to water stress for 5 days. Withholding water supply for 5 days resulting in some significant changes in a relative abundance of 98 proteins, 43 protein spots of the up-regulated and 55 for the down-regulated. In the controlled and the water-stressed plants for 6 days, 545 and 740 protein spots were attained from the peanut leaves. Water deficit stress conditions caused significant changes in a relative abundance of 71 proteins; 16 showing an increase in a relative abundance and 55 shown a decrease in a relative abundance. However, only 8 down-regulated and 2 up-regulated were identified (Table 2 and Fig 1). For proteomes of the peanut leaves under water stress for 5 days, the relative abundances of the down-regulated proteins were between 0.47 and 5.24 and the magnitudes of the changes were between 1.4 and 2.6. The relative abundances of the up-regulated proteins were 1.1 and 0.63 and the magnitudes of the changes were 1.5 and 1.3, respectively (Fig. 2 and Table 2). The down-regulated proteins include peptidyl-prolyl cis-trans isomerase FKBP4(D1), tRNA(Ile)-lysidine synthase, chloroplastic (D2), thioredoxin F-type, chloroplastic (D3), cytidine deaminase 7(D4), ABI3-like factor (ALF) domain class transcription factor (D5), nudix hydrolase 8 (D6), pentatricopeptide, a repeat super-family protein, putative (D7), ribulose-1,5-bisphosphate carboxylase/oxygenase, a large sub-unit (D8). Two up-regulated proteins are striated fibre assembling (SF-assembling, U1) and the Flap endonuclease 1(U2) where they are clearly shown in Table 2. On day 6 after

Table 1: Leaf water potential and relative water contents of the peanut plants under water stress conditions for 5 and 6 days compared with the plants of adequate water supply. Stress conditions took place at day 30 after seed emergence

Duration of water stress	Leaf water potential (MPa)	Relative water content (%)
5 days		
Adequate water supply	-0.20 (+0.02)	93.7 (+0.1)
Water stress	-0.42 (+0.03)	69.4 (+0.3)
6 days		
Adequate water supply	-0.23 (+0.02)	94.1 (+0.2)
Water stress	-1.11 (+0.04)	61.1 (+0.3)

Figures in the blankets represent Standard errors of means

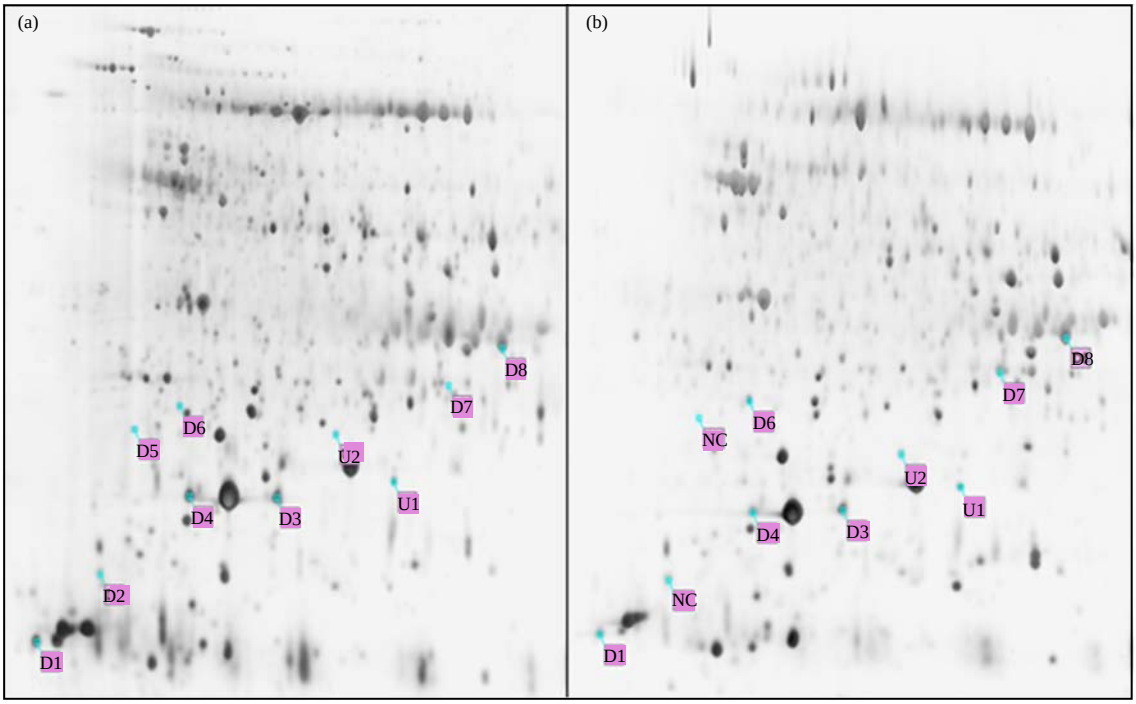

Fig. 1(a-b): Distributions of proteins in leaves of the Khon Kaen 4 peanut plants being regulated by water stress conditions. Proteins were separated using leaves of the peanut plants when under stress for (a) 5 days and (b) 6 days. D: Down-regulated, U: Up-regulated, NC: Non-change. The stress condition was taken place at day 30 after seed emergence

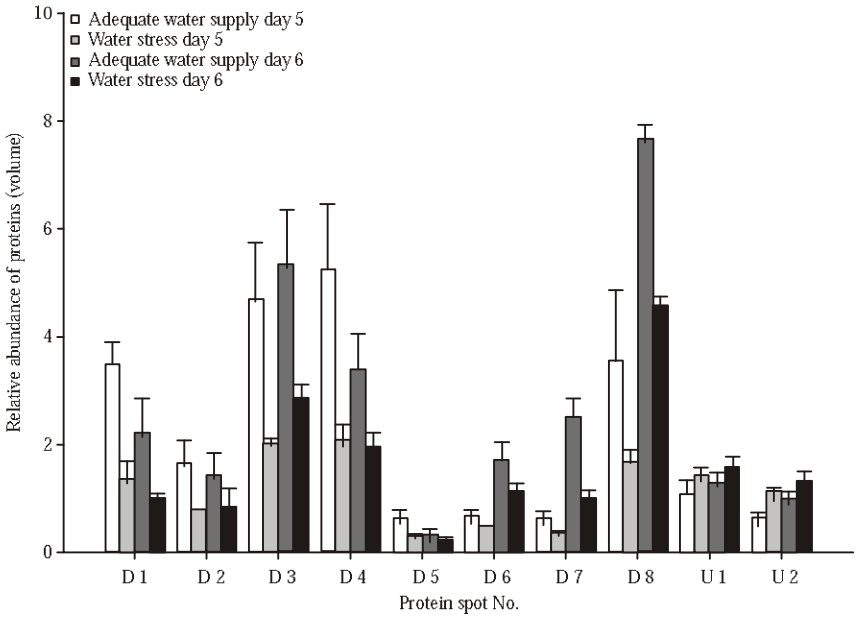

Fig. 2: Relative abundance of proteins of the peanut leaves being influenced by water stress for 5 and 6 days. Water stress commenced at day 30 after seed emergence. D: Down-regulated, U: Up-regulated. Vertical lines represent standard errors of means

Table 2: Spot numbers of the proteins in the peanut leaves as influenced by water deficit when stress conditions took place at day 30 after seed emergence

			Magnitude of change (days)						M_r (kDa)/pI	
Spot No.	Homologous proteins	Organism	5	6	Accession No[a]	Score[b]	Cov[c] (%)	Probability[d]	Theo[e]	Exp[f]
D1	Peptidyl-prolyl cis-trans isomerase FKBP4	*Triticum urartu*	2.6	2.2	EMS65417	85	31	0.005	23.03/8.23	28/4.32
D2	tRNA(Ile)-lysidine synthase, chloroplastic	*Chaetosphaeridium globosum*	2.1	NC	Q8M9Y1	69	29	0.004	40.65/9.9	33/4.62
D3	Thioredoxin F-type, chloroplastic	*Spinacia oleracea*	2.3	1.9	P09856	58	42	0.059	21.24/9.17	40/5.44
D4	Cytidine deaminase 7	*Arabidopsis thaliana*	2.5	1.7	Q9SU87	58	37	0.051	34.22/8.48	40/5.03
D5	ALF domain class transcription factor	*Malus domestica*	2.0	NC	ADL36572	74	26	0.065	47/4.77	74/27.34
D6	Nudix hydrolase 8	*Arabidopsis thaliana*	1.4	1.5	Q8L7W2	60	30	0.039	41.63/6.37	50/4.99
D7	Pentatricopeptide repeat superfamily protein, putative	*Theobroma cacao*	1.8	2.5	XP-007021301	76	15	0.047	84.56/7.14	51/6.24
D8	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	*Lantana camara*	2.1	1.7	AAF22242	79	43	0.022	53.49/6.2	57/6.49
U1	SF-assemblin	*Dunaliella bioculata*	1.3	1.2	P54214	60	23	0.037	31.51/5.41	41/5.98
U2	Flap endonuclease 1	*Ostreococcus tauri*	1.8	1.3	Q013G9	66	40	0.008	43.08/8.94	46/5.71

D: Down-regulated, U: Up-regulated , [a]Accession No: Accession number according to NCBI database, [b]Score, Individual ions, scores indicate identity or extensive homology ($p<0.05$), [c]Cov: Sequence coverage, [d]Probability: Significant homology, [e]Theo: Theoretical, [f]Exp: Experimental, Mr: Molecular weight, pI: Isoelectric point

the withholding water supply, only 7 proteins displayed significant changes. The relative abundance of the proteins was between 1.2 and 5.3 and the magnitudes of the changes were between 1.2 and 2.7 (Fig. 2 and Table 2). Two proteins, i.e., tRNA(Ile)lysidine synthase, chloroplastic (D2) and ALF domain class transcription factor (D5) did not change their relative abundance significantly when under water stress, whilst the remaining proteins displayed significant changes and the changes were the same as those found in the peanut leaves with the deprivation of the water for 5 days (Fig. 2 and Table 2).

DISCUSSION

Withholding water supply created severely water stresses in the Khon Kaen 4 peanut plants. Water stress appeared to be

moderate under 5-day duration but severely found at 6 days of water deprivation which were indicated by the LWPs and RWCs. Under different intensities of the water stresses, there were evidences shown on the differences in the patterns of protein expressions. Under a moderate water stress, there were 43 up and 55 down-regulated proteins. The up-regulated proteins may help to alleviate damages caused by the water deficit and the 55 down-regulated may be associated with the susceptibility of the Khon Kaen 4 peanut plants to water stress conditions. A severe water stress had caused a large decline in numbers of the up-regulated proteins down to 16 proteins, whilst maintaining 55 down-regulated proteins. A severe water stress may have caused a reduction in the up-regulated proteins that may act as the protection mechanisms against damages caused by the water deficit stress conditions.

Roles of the up-regulated proteins by water stress: The ROS causes the DNA base damage and the destruction of the deoxyrobose residues producing chain breaks (Bray and West, 2005). Flap endonuclease 1 (FEN1) occurred as a high-abundant protein with a relative abundance of 1.3 under water stress (Fig. 2). The flap endonuclease has its significant role in DNA repairing by the removal of the DNA damage and the alternate base-excision repair pathways. The DNA repair is essential for maintaining genome stability (Hosfield *et al*., 1998). The up-regulation of the FEN1 could possibly provide protective mechanisms against oxidative damages to the plant genomes under water stress conditions.

Striated Fibre assembling (SF assembling) is the major component of the Striated Microtubule-Associated Fibres (SMAFs) in the flagellar basal apparatus of the green flagellates (Lechtreck *et al*., 2002). Although structures and functions of the SMAFs have been well characterized in the Chlamydomonas reinhardtii (Silflow and Lefebvre, 2001), the roles of these proteins in the higher eukaryotic organisms are somewhat poorly understood. Since the SMAFs are associated with the structural constituents of the cytoskeletons, the proteins may function in mediating the plants in responding to the environmental factors where it provides mechanical support, barriers and the rapid transport routes (Wasteneys, 2004). An increase in the relative abundance of the SF assembling may help to trigger the rapid responses against water stress and to maintain a cellular integrity under water stress and also to alleviate damages from the cellular dehydration.

Down regulated proteins and protein synthesis: ALF domain class transcription factor (D5) is a B3 domain-containing the transcription factor that activates specific ABA-dependent gene expressions. The ALF potentiates chromatin for transcription by mediating histone modifications (Chinnusamy *et al*., 2008). The ABA triggers the assembly of the ABA signaling cascade components leading to the recruitment of the RNA Pol II and GTFs. Both the ALF and ABA are required for specific gene expressions (Ng *et al*., 2006). Water stress may have some adverse effects on specific gene expression due to down-regulation of the ALF domain class transcription factor (D5). However, under a severe water stress condition, this protein did not display a significant change suggesting an acclimated response of the protein to its progressive water stress.

Putative Pentatricopeptide Repeat (PPR) proteins (D7) are in one of the largest families in the plants. The PPR proteins are involved in the RNA processing including editing, maturation, stability, and translation in the plant organelles. The PPR proteins may act as the transacting factors and facilitate the recruitment of other factors for the RNA processing and translation (Saha *et al*., 2007). The down regulation of the PPR protein may perturb RNA processing and translation where it could be critical for the cellular processes.

Chloroplastic tRNA(Ile)-lysidine synthase (D2) converts both the codon specificity of the tRNA(Ile) from the AUG to AUA and its amino acid specificity from methionine to isoleucine (Suzuki and Miyauchi, 2010). The enzyme will definitely modify the amino acid composition of the targeted proteins. However, the involvement of this enzyme under drought stress responses is poorly documented. This protein did not change a relative abundance under a severe water stress.

Cytidine deaminase catalyzes the hydrolyric deamination of cytidine into uridine. The higher plants use cytidine deaminase as editing systems in the conversion of the Cs to the Us in the edited RNAs. In the RNA editing, it occurs mainly in the mitochondria and plastids (Shikanai, 2006). The RNA editing is essential as to express functional proteins but it can also create an initiation or stop codon or remove a stop codon. The editing occurs in the introns and the untranslated regions which may play a significant role in increasing transcript stability (Chateigner-Boutin and Small, 2010; Grennan, 2011). A decline in the abundance of cytidine deaminase 7(D4) may decrease the activity of the RNA editing leading to the production of the non-functional proteins that could be detrimental to cellular processes.

The FK506-binding proteins (FKBPs) belong to the large family of the peptidyl-prolyl cis-transisomerases (PPIases), which catalyses protein folding by accelerating the slow step of the cis-trans isomerisation of the peptidyl-prolyl bonds. The plants of the FKBP also function as molecular chaperone, independently of its peptidylprolyl cis-trans isomerase (Kurek *et al*., 2002). The expression of the genes encoding the FKBPs is regulated by the water stress phenomena (Yu *et al*., 2012). The down-regulation of the peptidyl-prolyl cis-trans isomerase FKBP4 (D1) may adversely affect protein folding during water stress condition and that the damaged proteins could not be recovered under the water stress condition where it rendering this peanut variety to be susceptible to water stresses.

Photosynthesis and oxidative stressunder water stress conditions: Photosynthetic capacity of plants invariably decreases during the drought stress conditions (Chaves *et al*., 2009). Ribulose-1,5-bisphosphate carboxylase/oxygenase

(Rubisco) of a large sub-unit (LS-D8) is a key enzyme responsible for the CO_2 fixation during the photosynthetic process. This protein presents in a large amount with a relatively abundance of 3.5 and 7.6 under an adequate water supply on days 5 and 6 after imposing water stress, respectively. The relative amount of the protein decreased by 2.1 and 1.7 folds under the withholding of water supply for 5 and 6 days, respectively (Fig. 2 and Table 1). The effect of drought conditions on the amount of the Rubisco is somewhat contradictory. Withholding water supply for 3, 6 and 9 days resulted in the progressive declines in photosynthetic rates but have caused to increase amount of the Rubisco LS (Kamal *et al*., 2013). However, the content of the Rubisco LS decreased to 50-75% in the drought stressed leaves of both drought-tolerant and drought sensitive varieties (Demirevska *et al*., 2009). A reduction in the amount of the Rubisco LS in this current experiment may suggest that a decline in photosynthetic capacity of the peanut plants under water stress may be associated with the drought susceptibility of the peanut plants.

Chloroplastic thioredoxins (TRX) F-type must have involved in the regulation of the key carbon-fixation enzymes that are mostly inactive in the dark and activated by the TRXs underillumination (Schurmann and Jacquot, 2000). The lower abundance of the chloroplastic thioredoxin F-type (D3) in the peanut leaves may be attributable to the decreased amount of the activated key enzymes in the carbon fixation process and the photosynthetic capacity of leaves (Yoshida *et al*., 2014).

Nudix (nucleoside diphosphates linked to moiety X) hydrolases play a vital role in the cellular homeostasis by catalyzing the hydrolysis of a variety of nucleoside diphosphate derivatives. ZmNUDX8 (*Zea mays* NUDX8) and AtNUDX2 (*Arabidopsis thaliana* NUDX2) belong to the ADP-ribose pyrophosphatase (ADPR) sub-family (Huang *et al*., 2012). The AtNUDX2 expression could have been induced by salt and drought stress conditions. An over expression of the AtNUDX2 resulted in enhancing tolerance to the oxidative stress by maintaining the NAD^+ and ATP levels (Ogawa *et al*., 2009). The down-regulation of the Nudix hydrolase 8 (D6) in the peanut leaves under water stress conditions may have lowered the capacity of the antioxidant systems to cope up with any oxidative stress conditions.

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