Differential expression of leaf proteins in four cultivars of peanut (*Arachis hypogaea* L.) under water stress

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Abstract

Drought is a major constraint to the productivity of many crops affecting various physiological and biochemical processes. Seventy percent of the peanuts are grown in semiarid tropics that are frequently prone to drought stress. So, we analyzed its effect in 4 cultivars of peanut, with different degrees of drought tolerance, under 10 and 20 days of water stress using two-dimensional gel electrophoresis and mass spectrometry. A total of 189 differentially expressed protein spots were identified in the leaf proteome of all the 4 cultivars using PD Quest Basic software; 74 in ICGV 91114, 41 in ICGS 76, 44 in J 11 and 30 in JL 24. Of these, 30 protein spots were subjected to in-gel trypsin digestion followed by MALDI-TOF that are functionally categorized into 5 groups: molecular chaperones, signal transducers, photosynthetic proteins, defense proteins and detoxification proteins. Of these, 12 proteins were sequenced. Late embryogenesis abundant protein, calcium ion binding protein, sucrose synthase isoform-1, 17.3 kDa heat shock protein and structural maintenance of chromosome proteins were overexpressed only in the 15 and 20 days stressed plants of ICGV 91114 cultivar while cytosolic ascorbate peroxidase was expressed with varying levels in the 10 and 20 days stressed plants of all the 4 cultivars. Signaling protein like 14-3-3 and defense proteins like alpha-methyl-mannoside-specific lectin and mannose/glucose-binding lectins were differentially expressed in the 4 cultivars. Photosynthetic protein like Rubisco was down-regulated in the stressed plants of all 4 cultivars while Photosystem-I reaction center subunit-II of chloroplast precursor protein was overexpressed in only 20 days stressed plants of ICGV 91114, ICGS 76 and J11 cultivars. These differentially expressed proteins could potentially be used as protein markers for screening the peanut germplasm and further crop improvement.

Keywords Peanut · Water stress · 2-DE · PMF · MALDI-TOF · Differential expression

Abbreviations

LEA-1	Late embryogenesis abundant protein-1
CalM42	Calcium ion binding protein
Susy-1	Sucrose synthase isoform-1
SMC-1	Structural maintenance of chromosome-1
APX-1	Ascorbate peroxidase-1

PS I	Photosystem-I reaction center subunit-II of chloroplast precursor protein
10 DS	10 days water-stressed plants
20 DS	20 Days water-stressed plants
MALDI-TOF	Matrix-assisted laser desorption/ionization-time-of-flight

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Introduction

Peanut is the most important crop for edible oil production and cultivated heavily in almost all the tropical and subtropical countries. The productivity levels of this crop are low due to a number of abiotic factors among which drought is the single largest factor affecting various physiological and biochemical processes (Jaleel et al. 2008; Farooq et al. 2009). Plants start responding and adapting to drought stress at various levels such as morphological, anatomical

and molecular level (Chaves et al. 2003; Timperio et al. 2008). Response to drought stress also depends on the type of species and genotypes. The major molecular response to drought stress is altered gene expression in relation to various pathways associated with stress perception, signal transduction and synthesis of stress related compounds (Ramanjulu and Bartels 2002). Plants also respond to stress by synthesizing novel proteins or up-regulating protein expression (Komatsu and Hossain 2013).

Proteomic analyses of various crops revealed numerous drought-stress responsive proteins involved in photosynthesis, oxidative stress response, redox regulation, signal transduction, protein folding and secondary metabolism in rice (Salekdeh et al. 2002; Ke et al. 2009), wheat (Faghani et al. 2015), sunflower (Castillejo et al. 2008), soybean (Kunert et al. 2016). Despite the agronomic and economic importance of peanut, very little is known about its molecular adaptive responses to drought (Luo et al. 2005; Kottapalli et al. 2009). Many biochemical processes in a cell are regulated by protein–protein interactions, post-translational modifications and enzymatic activities that cannot be identified by gene expression studies alone (Morris et al. 2007). Therefore, proteomics is a powerful tool to study the molecular response of plants to biotic and abiotic stresses (Kottapalli et al. 2009). Despite the development of high-density oligonucleotide microarray using 49,205 ESTs available in the public domain, a very little information is known regarding the molecular changes due to drought stress in peanut (Payton et al. 2009). Through genomic approach, Jain et al. (2001) identified 43 differentially expressed peanut transcripts responsive to drought (PTRD). Among them, 12 were completely suppressed under prolonged drought conditions, 2 were down-regulated and 2 up-regulated in response to drought stress. Guo et al. (2006) reported rapid induction of phospholipase D alpha (PLD) gene in the drought-sensitive lines under drought stress than in the drought-tolerant lines. Drame et al. (2007) investigated the involvement of phospholipids, proteases and LEA proteins in conferring drought tolerance in peanut plants and reported that accumulation of putative PLD and LEA proteins leads to increased drought tolerance. Devaiah et al. (2007), using differential display of mRNA transcripts, identified the increased expression of two genes encoding *Arachis hypogaea* serine-rich protein (*AhSrp*) and *Arachis hypogaea* leucine-rich protein (*AhLrp*) under drought stress. Using differential mRNA display, Govind et al. (2009) showed that nearly 700 genes were enriched in subtractive cDNA library in response to gradual water stress. These 700 candidates include genes encoding kinases, transcription factors and phosphatases, late embryogenesis abundant proteins, heat shock proteins, DnaJ like proteins, aldehyde reductase, proline rich protein and defensins, and phytohormones such as brassinosteroids, auxin and cytokinin responsive genes. Pruthvi et al. (2013) carried out expression analysis of few drought stress responsive ESTs from cultivated peanut and

reported the association of genes like cyclin T, proline amino peptidase and choline kinase to drought tolerance.

In peanut, proteomic analysis identified differential expression of diverse seed storage proteins in drought-tolerant and susceptible genotypes besides characterization of arachin and methionine-rich proteins from cultivated peanut (Basha and Roberts 1981; Basha et al. 2007). Katam et al. (2007) evaluated genetic diversity in 200 peanut cultivars by studying their seed and leaf protein contents and their response to water stress. Kottapalli et al. (2009) studied the differential expression of leaf proteins during reproductive growth stage under drought stress, in U.S. peanut mini core collections, at maturity phase. They identified 49 non-redundant proteins and reported the overexpression of lipoxygenase and 1L-myo-inositol-1-phosphate synthase proteins in tolerant genotypes under water-deficit stress. Some of the important proteins playing a significant role in drought tolerance include acetyl-CoA carboxylase, several lectins and proteins involved in cellular detoxification, signal transduction and energy metabolism, etc. A reference leaf proteome map was developed in drought-tolerant peanut cultivar, Vemana by Katam et al. (2010). Recently, Katam et al. (2016) have done a comparative study on proteomic profiles of drought-tolerant and susceptible cultivars and also identified 42 unique protein interactions in tolerant cultivar and 20 interactions in susceptible cultivar. They reported that proteins like glutamine ammonia ligase, chitin class II, actin isoform B, and beta tubulin were unique to tolerant cultivar while serine/threonine protein phosphate PP1, choline monooxygenase, peroxidase 43, and SNF1-related protein kinase regulatory subunit beta-2 were not unique but induced upon drought stress in drought-tolerant cultivar. However, the data on protein expression in peanut under drought stress is still limited. Therefore, this study was undertaken with an aim of identifying and characterizing drought stress responsive proteins through ‘proteomics approach’ by subjecting 4 high yielding and locally adaptable peanut cultivars with varying degrees of drought tolerance, to different stress periods. The main aim of this study is to gain an understanding on the molecular basis of differential response of cultivars to water stress periods for the differential expression of proteins, thereby helping to improve productivity levels by developing high-yielding genotypes that can survive drought stress.

Materials and methods

Plant material

Four varieties of peanut (*Arachis hypogaea* L.), ICGV 91114, ICGS 76, J 11 and JL 24, with varying degrees of drought tolerance were obtained from the International

Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, Andhra Pradesh, India. ICGV 91114 is a high yielding (2.5–3.0 ton/ha), early maturing (90–95 days) bunch variety with an ability to withstand prolonged drought spells, tolerant to mid-season and end-of-season drought. ICGV 76 is another high yielding (1.3–1.8 ton/ha) virginia bunch variety that matures in 120 days and has a good recovery from mid-season drought. J 11 is a spanish bunch type adaptable under a wide range of agro-climatic conditions while JL 24 is also a high yielding (1.5–2.0 ton/ha) spanish bunch variety that matures in 90–95 days and is one of the most popular national varieties sown in areas where end-season drought is common.

Imposition of drought stress

The experiment was laid out in a completely randomized design with three replications for each variety and the seedlings/plants were maintained in the net house covered with water proof sheets during the experiment at the plant Genetics experimental farm, Department of Genetics, Osmania University, Hyderabad. Three independent pots were maintained for each control and stress treatment for each variety, considering each pot as a replication, with 4 seeds per pot. The seeds were sown at a depth of 4 cm in a pot measuring 225 mm height and 450 mm diameter. The pots were filled with a mixture of soil: sand at 2:1 ratio and nutrients like NPK fertilizers were added at a ratio of 2:3:2 for the growth of seedlings. They were watered regularly thrice a week for 20 days until the water stress was imposed. Drought was imposed by withholding water to the 20-day-old seedlings for 10, 15 and 20 days to ICGV 91114 while 10 and 20 days to other three cultivars, maintaining their respective controls. Fully expanded fresh leaf samples were collected at random from each replicate of the stressed plants of all the four varieties along with their respective controls on 31st and 41st days, respectively, quickly frozen in liquid nitrogen and stored at -80°C prior to protein extraction.

Relative water content (RWC)

The RWC was estimated in 10, 15 and 20 days water stressed plants along with their respective controls in all the 4 cultivars. After recording the fresh weight of the collected leaves from 31st, 36th and 41st day-old seedlings, they were stored overnight in deionised water, in a refrigerator, at 4°C and the next day, the turgid weight of the blotted leaves was recorded. Later, the dry weight of leaves, incubated in hot air oven at 60°C for 24 h, was recorded. The leaf RWC was then measured and calculated as per the method proposed by Sharp et al. (1990) using the formula, $\text{RWC}(\%) = [(FW - DW)/(TW - DW)] \times 100$, where FW is the fresh weight, DW is the dry weight and TW is the turgid

weight. The leaf RWC is expressed as a percentage of fully turgid water content.

Protein extraction

Two grams of fresh leaf tissue (pooled from 3 replicates) of each variety was ground to fine powder in chilled mortar and pestle with liquid nitrogen and homogenized twice with 20 ml of cold acetone containing 10% trichloroacetic acid (TCA) and 0.07% β -mercaptoethanol (β -ME) for 30 s. This homogenate mixture was incubated overnight at -20°C and then centrifuged at 20,000 g for 30 min. The supernatant was discarded and the pellet was homogenized again with fresh acetone containing 10% TCA and 0.07% β -ME. This pre-protein extraction procedure was repeated 3–4 times until all the pigments from the leaf tissue were removed. Later, this pellet was homogenized with cold acetone containing only 0.07% β -ME without TCA and centrifuged at 10,000 g for 20 min. This step was repeated twice and the pellet was air dried overnight.

Preparation of protein extract for 2-DE

The total leaf proteins were extracted by thorough vortexing and homogenization of the pellet in 5 ml of protein extraction buffer consisting of 8.8 M urea, 2.0 M thiourea, 4% CHAPS (3-[3-cholamidopropyl dimethylammo-o]-1-propanesulfonate), 20 mM Di-thiotheritol (DTT), 10% protease inhibitors cocktail and 0.01% biolytes. This homogenate was incubated at room temperature for one hour, centrifuged at 20,000 g for 30 min and filtered through a 0.45 μm filter. This protein extraction was repeated thrice and all the supernatants were pooled and the protein was estimated using Bradford assay (Bradford 1976).

Two-dimensional polyacrylamide gel electrophoresis (2-DE)

The above purified total leaf proteins were resolved by 2-DE in two steps: in the first dimension by isoelectric focusing (IEF) and in the second dimension by SDS-PAGE. Briefly, 250 μg of leaf protein was loaded onto 7 cm IPG strips (Bio-Rad) with pH 4–7 and kept overnight for rehydration. IEF of proteins was performed with 50 mA/strip with the following program: Step-I: 250 V for 20 min. at a linear slope, Step-II: 4000 V for 2 h, Step-III: 4000 V, at a rapid slope for 10,000 Vh, and Step-IV: 4000 V at a linear slope for 30 min. Later, these strips were equilibrated with an equilibration buffer-I (containing 6 M urea, 2% (w/v) SDS, 20% (v/v) glycerol, 0.375 M Tris-HCl (pH 8.8) and 2% (w/v) DTT) for 15 min and then for another 15 min with equilibration buffer-II (containing 6 M urea, 2% (w/v) SDS, 20% (v/v) glycerol, 0.375 M

Tris–HCl (pH 8.8) and 2.5% (w/v) iodoacetamide). At the end of equilibration, the strips were loaded onto the SDS-PAGE for the second dimension. In the second dimension, SDS-PAGE of all the protein samples, along with a pre-stained broad range SDS-PAGE marker (Bio-Rad), was performed using 12% (w/v) polyacrylamide gels with 5% (w/v) stacking gels. Electrophoresis was carried out in a Bio-Rad PROTEAN unit at a constant current of 20 mA/gel in 1-X Tris–Glycine electrophoretic running buffer. After electrophoresis, the gels were fixed in a mixture of 40% methanol and 10% glacial acetic acid, stained with 0.25% colloidal Coomassie Brilliant Blue R-250 (CBB) and de-stained in a mixture of 40% methanol and 10% glacial acetic acid solution.

Image analysis

Protein profiles in the CBB-stained polyacrylamide gels were scanned as digitized images using GS-710 Calibrated Imaging Densitometer (Bio-Rad) with a scan density of 42.3×42.3 and saved as tagged image file formats. These protein spots were quantified using PD Quest Basic software version 8.0.1 (Bio-Rad) which includes spot detection, measurement, background subtraction and matching. The protein spots on the polyacrylamide gels obtained from control plants of all the 4 cultivars were differentiated into faint, small and large spots. These control gels were used as reference gels for comparison of spots on the polyacrylamide gels obtained from their respective stressed plants. The quantitative variations in the intensity of protein spots due to CBB staining of the polyacrylamide gels were corrected using normalization parameters and thus the gaussian images were created. These Gaussian images exhibiting the matching between the protein spots of controls and their respective stressed plants, of each of the 4 varieties, were retained and rest of the unmatched spots were removed using spot editing tool. Protein spots across the replicate gels of the control and stressed plants within a variety were subjected to auto spot matching by the ‘classic match tool’ in all the 4 cultivars. The unmatched spots on the member gels of the stressed plants were added to the reference gel (control gel image). Qualitative analysis, to know the presence of the spots, and quantification, of spots to know their intensity, was performed by employing the ‘analysis set manager’ tool. For quantification of protein spots in a set of gels within a variety, the outside limits were set as 0.5 and 1.5. The spots showing expression levels < 0.5 were considered as down-regulated > 1.5 as more abundant and between these limits as unchanged or normal spots. On the basis of matching, differential spots were selected and analyzed as described below. Three replicates gels were analyzed for each sample to ensure reproducibility.

In-gel trypsin digestion

The CBB stained 2 DE gels were washed several times with ultra-pure water and protein spots of interest were excised from gels. These spots were de-stained in a solution containing 200 mM NH_4HCO_3 in 40% acetonitrile (ACN) for 30 min. at 37 °C and dried using speedvac for 15 min. These dry spots were subjected to in-gel trypsin digestion by the addition of 0.5 µg trypsin and reaction buffer consisting of 40 mM NH_4HCO_3 and 9% ACN and digestion was allowed to continue at 37°C overnight. After digestion, the peptide samples were extracted in 0.1% trifluoroacetic acid (TFA) and 50% ACN and stored at -20°C for MALDI-TOF analysis.

Mass spectrometry analysis

The trypsin digested peptides were loaded into a constricted GELoader tip (Eppendorf, Hamburg, Germany) packed with POROS R2 chromatographic resin (Perseptive Biosystems, Framingham, MA) with 5 mm porosity for desalting and concentration. The columns were equilibrated with 20 ml of 5% formic acid (FA) into which the digested samples were added. Later, the bound peptides were washed with 20 ml of 5% FA and eluted directly onto the MALDI target with 0.5 ml of CHCA solution (5 mg/ml in ACN, 0.1% TFA, 70:30 v/v). These samples were analyzed in an Applied Biosystems 5800 MALDI-TOF Proteomics Analyzer. The instrument was equipped with a nitrogen laser and operated in a positive-ion delayed extraction reflector mode. External calibration was performed using standard peptide/protein mixture. Usually, 250 individual spectra of each spot were averaged to produce a mass spectrum. The atmospheric air was used as collision gas to fragment the peptides and obtain the spectra. Peptide fragmentation was performed using collision-induced dissociation (CID) and 50 laser shots from five sample positions were summed up for each parent ion.

Protein identification and database search

Identification of proteins was performed by searching against the National Center for Biotechnology Information non-redundant (NCBI nr) and SWISS-PROT databases using Mascot software (<http://www.matrixscience.com>) (Perkins et al. 1999, Eriksson et al. 2000) with Viridiplantae (green plants) as the taxonomic category. The MASCOT search compares the experimental data to all the sequences in a database and returns the list of hits with decreasing scores, a measure of reliability of identification. The following parameters were used for database search with MALDI-TOF PMF data: (NCBI nr 20070216, 4626804 sequences, 1596079197 residues; Taxonomy Viridiplantae, 186963 sequences), trypsin as digesting enzyme, 2 missed cleavages allowed,

carbamido-methylation, methionine-oxidation and deamidation (NQ) (variable modifications), monoisotopic mass, peptide mass tolerance at ± 100 ppm, unrestricted protein mass, 1 + peptide charge state. The search scores were represented as probability-based Mowse score $-10 \times \log(P)$, where P is the probability that an observed match was a random event. Protein scores greater than 65 were considered significant ($p < 0.05$) in NCBI and scores > 56 were considered significant in SWISS-PROT database. For a positive identification in MALDI TOF-MS, the peptide score should exceed or equal to minimum significant score. For database search with MS/MS spectra, the following parameters were used: (CDS combined KBMS5.0.20050302, 1967674 sequences, 672312456 residues; Taxonomy Viridiplantae, 177633 sequences), trypsin enzyme, carbamido-methylation, methionine-oxidation, deamidation (NQ) (variable modification), monoisotopic mass value, unrestricted protein mass, peptide mass tolerance at ± 1.0 Da, unrestricted protein mass, +1, +2, +3 peptide charge state with one missed cleavage allowed. Proteins were considered detected if they were identified by more than two peptides per spot.

Predicting sub-cellular localization and functional annotation

Gene ontology was predicted for all the 30 proteins identified through PMF, by searching the identified protein ID in TargetP program (www.cbs.dtu.dk/services/TargetP) (Emanuelsson et al. 2007).

Statistical analysis

The data for RWC were recorded and analyzed through two-way analysis of variance (ANOVA) and the values were expressed as mean and standard error. The significance of the treatment effects was tested at 5% probability level ($P = 0.05$) using Tukeys test, which is one of the Post Hoc multiple comparisons of Two-way ANOVA of Windostat version 8.5.

Results and discussion

After withholding water to 20-day-old seedlings at vegetative phase, ICGV 91114, ICGS 76 and J 11 cultivars started showing the symptoms of wilting after 15 days of water stress while JL 24 variety wilted after 10 days of water stress. Hence, the study was conducted under mild (10 days) and severe (20 days) water stress conditions while in ICGV 91114 cultivar, the leaf proteome was studied at moderate (15 days) stress also to investigate the effect of drought stress on differential expression of proteins in the 4 cultivars (Fig. 1 a, b, c, d).

Effect of drought stress on relative water content (RWC) of leaf

Decrease in RWC is one of the early symptoms manifested during drought stress and is considered to be the best integrated measure of plant water status that represents variations in water potential (WP), turgor potential (TP) and osmotic adjustment (OA) in plant tissues (Rampino et al. 2006; Sanchez-Rodriguez et al. 2010). In our experiment, the 4 varieties evaluated for their drought tolerance exhibited significant reduction in RWC with an increase in the duration of water stress from 10 to 20 days ($p < 0.01$). The highest RWC was observed in ICGV 91114, followed by ICGS 76, J 11 and JL 24 in the 10, 15 and 20-day stressed plants including the controls (Fig. 2). Groundnut is relatively a drought-tolerant crop with improved water use efficiency mechanisms that allow the plant to withstand water stress for certain period of time (Nautiyal et al. 2002). RWC was reported to be one of the best indicators of plant water status in maize (Ritchie et al. 1990), wheat (Valentovic et al. 2006) and tomato (Sanchez-Rodriguez et al. 2010) for separating tolerant and sensitive cultivars. The ability of ICGV 91114 to maintain high RWC might be a resistant mechanism which is a result of more osmotic regulation maintained by accumulation of osmolytes (Padmavathi and Rao 2013). On the contrary, JL 24 variety showed reduction in RWC to a greater extent, attributing to its inability to accumulate osmolytes for longer periods leading to decreased osmotic potential, thereby losing its turgor making it a susceptible variety.

Two-dimensional (2-DE) gel analysis of leaf proteins

On 2-DE gels, the total leaf proteins resolved in the range of 12–100 kDa between 4 and 7 pH with the majority of proteins separating between 20 and 40 kDa and 5.0–6.8 pH. Some proteins with molecular weights > 50 kDa did not show complete resolution resulting in clusters of multiple protein spots which is due to very similar molecular weights and a slight difference in pI values. Most of these clusters were concentrated between 4 and 7 pH with molecular weights in the range of 50 and 75 kDa. Quantification of protein spots on 2-DE gels, using PD Quest software, revealed the presence of 189 differentially expressed protein spots in the stressed plants of 4 cultivars against their controls. In ICGV 91114, a total of 74 differential protein spots were observed. Of them, 20 spots were seen in 10 DS (8 more abundant, 7 down-regulated and 5 new), 5 in 15 DS (3 more abundant and 2 new) and 14 in 20 DS plants (9 more abundant, 4 down-regulated and 1 new). A total of 14 protein spots were found in common among all the stressed plants, however, with variable levels of expression. 6 protein spots (spot No.23, 33, 34, 35, 51 and 54) were common between

Fig. 1 **a** 10-, 15- and 20-day water stressed plants of ICGV 91114 along with their controls. Drought was induced to the 20-day-old peanut seedlings at vegetative phase under pot culture. The plants could withstand drought up to 15 days of water stress and then started showing the symptoms of wilting. **b** 10-, 15- and 20-day water-stressed plants of ICGS 76 along with their controls. Drought was induced to the 20-day-old peanut seedlings at vegetative phase under pot culture. The plants could withstand drought up to 15 days of water stress and then started showing the symptoms of wilting. **c** 10-, 15- and 20-day water-stressed plants of J11 along with their con-

trols. Drought was induced to the 20-day-old peanut seedlings at vegetative phase under pot culture. The plants could withstand drought up to 10 days of water stress and then started showing the symptoms of wilting in 15-day stressed plants and completely wilted in 20-day stressed plants. **d** 10-, 15- and 20-day water-stressed plants of JL24 along with their controls. Drought was induced to the 20-day-old peanut seedlings at vegetative phase under pot culture. The plants could withstand drought up to 10 days of water stress and then started wilting completely in 15-day stressed plants and drying up in 20-day stressed plants

10 and 15 DS, 14 spots were common between 15 and 20 DS while only protein spot No. 10 is common between 10 and 20 DS (Fig. 3 & Supplementary Table 1). In ICGS 76, a total of 41 protein spots exhibited differential expression. In 10 DS, 17 spots were expressed more abundantly and 12 were down-regulated. Interestingly, 5 new spots; 19,20,21,22,23 appeared while spot No. 29 disappeared. Apart from the 26 common spots (spot No. 1–11, 13, 16, 17, 19, 20, 22,

24, 25, 26, 28, 30, 34, 38, 40 and 41 in 10 DS), in 20 DS, 9 protein spots were expressed additionally that includes 4 more abundant (spot No. 34,35,36 37), 2 down-regulated (spot No. 38, 41) and 3 new proteins (spot No. 31, 32, 33) (Fig. 4 and Supplementary Table 2). In J11 cultivar, 44 protein spots showed differential expression, out of which 30 spots were expressed in 10 DS that include 9 spots that were down-regulated, 12 spots expressed more abundantly,

Fig. 2 Relative water content in 4 cultivars of peanut subjected to 10, 15 and 20 days of water stress. Four seeds from each variety were sown in pots filled with a mixture of soil and nutrients in a green house. Pots were watered regularly thrice a week for 20 days until the water stress was imposed. Drought was imposed by withholding water to the 20-day-old seedlings for 10, 15 and 20 days. At the end of experiments, the relative water content of leaves was estimated as described in methods. The bars represent mean $\pm$ SD and the bars that do not share common superscript differ significantly ($p < 0.05$)

7 new spots (15,17,19,23, 27, 28 and 30) and 2 spots (25 and 26) that disappeared. Both 10 and 20 DS showed 14 protein spots in common. There was no change observed in the expression of Spot No. 32, 33, 34, 38, 40, 41 and 44 in 10 DS in comparison with controls while in 20 DS, these spots were expressed more abundantly and also all the new spots observed in 10 DS did not show any expression in 20 DS (Fig. 5 and Supplementary Table 3). In JL 24, of the 30 protein spots, 28 spots were expressed in 10 DS (12 more abundant, 16 down-regulated and 2 new) and 2 spots in 20 DS. Among these 30 spots, 14 spots were common between 10 and 20 DS, however, with different levels of expression (spot No. 5,6, 12, 13 and 25 were more abundant in 10 DS while down-regulated in 20 DS. Similarly, spot No. 1,2,3,7,8,9,10, 15, 17, 18, 19, 20, 21, 26, 27 and 30 were expressed only in 10 DS and did not show any sort of expression 20 DS (Fig. 6 and Supplementary Table 4).

Fig. 3 The 2D gels showing the leaf proteome of peanut cultivar ICGV 91114. The leaf protein extracted from drought stressed and respective control plants from 10 (a), 15 (b) and 20 days (c) was sub-

jected to 2D-gel electrophoresis as described in methods. The images were digitized and quantified densitometrically

Table 1 Peptide mass fingerprinting (PMF) of differentially expressed protein spots in ICGV 91114

Sp. No.	Exptl. M.Wt.	Theo. M.Wt.	Homologous protein	Organism	Biological Process	Molecular function	Cellular component	Mascot score	Accession no.
1	56.0	52.6	RUBP carboxylase large chain	<i>Cadellia pentastylis</i>	Photorespiration/reductive pentose-phosphate cycle	Mono-oxygenase/RUBP carboxylase activity	Chloroplast/plastid	519	gil1351139
17	16.2	16.2	PR 10 protein	<i>Arachis hypogaea</i>	Defense response/response to biotic stimulus	–	–	65	gil52547774
50	30.0	100.5	Unnamed protein product	<i>Oryza sativa</i>	Carbohydrate metabolic process	Hydrolase activity	–	74	gil8099130
38	19.5	92.4	Sucrose synthase isoform 1	<i>Daucus carota</i>	Biosynthetic/sucrose metabolism	Sucrose synthase/Glycosyl transferase	–	82	gil1351139
38	19.5	140.8	Structural maintenance of chromosome 1	<i>Arabidopsis thaliana</i>	DNA repair/chromosome condensation	ATP binding	Nucleus	73	gil45594277
39	26.5	49.2	Putative serine/threonine protein kinase	<i>Oryza sativa</i>	Signaling	ATP binding/protein serine/threonine kinase activity	Plasma membrane	51	gil30017556
40	14.4	14.1	Unknown protein	<i>Arabidopsis thaliana</i>	–	–	–	80	gil30695529
40	14.4	14.2	Probable myosin heavy chain	<i>Arabidopsis thaliana</i>	–	–	–	79	gil25408221
9	31.5	124.8	Gamma response I protein	<i>Arabidopsis thaliana</i>	Signaling	Sequence specific DNA binding TF	–	70	gil4678941
9	31.5	20.4	Ubiquitin conjugating enzyme	<i>Arabidopsis thaliana</i>	Post replication repair	Acid-amino acid ligase activity/protein binding	Cytosol	66	gil18403085
47	41.5	66.2	Hypothetical protein	–	–	–	<i>Oryza sativa</i>	69	gil51038186
48	19.5	22.3	OSJNBa0029C04.4	DNA integration/RNA dependent DNA replication	RNA binding/ribonuclease H activity	–	<i>Oryza sativa</i>	92	gil38346291
53	21.5	36.8	Late embryogenesis abundant protein	–	–	–	<i>Pisum sativum</i>	66	gil56709428
55	21.0	76.9	F3F9.11 protein	Detoxification/Regulation of growth	Enzyme binding/glutathione transferase activity	Cytoplasm/Nucleus	<i>Arabidopsis thaliana</i>	76	gil8052534
44	21.5	6.5	Stress-induced protein KIN1	Stress response	–	–	<i>Arabidopsis thaliana</i>	50	KIN1_ARATH
74	33.0	47.5	26S protease regulatory subunit 7	Protein catabolic process	ATP binding/nucleoside triphosphatase activity	Cytosol/Plasma membrane/proteosome	<i>Arabidopsis thaliana</i>	60	PRS7_PRUPE
70	26.5	21.3	Endoribonuclease Dicer homolog (EC 3.1.26.-)	Plant defense/RNA mediated gene silencing	ATP binding/protein binding/metal ion binding	Cytoplasm/Nucleus	<i>Arabidopsis thaliana</i>	73	DICER_ARATH

Table 1 (continued)

Sp. No.	Exptl. M.Wt.	Theo. M.Wt.	Homologous protein	Organism	Biological Process	Molecular function	Cellular component	Mascot score	Accession no.
59	24.0	43.4	GSK-like kinase	-	ATP binding/protein serine/threonine kinase activity	-	<i>Triticum aestivum</i>	67	gil21745456
43	17.3	17.3	17.3 kDa class I heat shock protein	Stress response	-	Cytoplasm	<i>Glycine max</i>	70	gil123534
24	26.5	84.9	Kinesin-3 (Kinesin-like protein C)	Cell division/mitosis/microtubule based movement	ATP binding/ATPase activity/Microtubule binding/Microtubule motor activity	Cytoplasm/Microtubule	<i>Arabidopsis thaliana</i>	66	ATK3_ARATH

Sp.No., spot number, Exptl. M.Wt., experimental Mol. Wt., Theo. M.Wt., theoretical M.Wt.

Peptide mass fingerprinting (PMF) analysis

Based on the fold change and visual scoring, 30 differentially expressed protein spots; 17 from ICGV 91114, 6 from ICGS 76 and 7 from J 11 were selected and subjected to trypsin digestion to carryout PMF analysis. These digested peptides were injected to MALDI-TOF for obtaining the mass spectra (MS) of each protein. The MASCOT search in NCBI database for comparing the mass of these peptides with that of the theoretical mass of proteins is deposited in the taxonomy; Viridiplantae (green plants) resulted in the identification of 30 proteins (Tables 1, 2 and 3). Of these, 28 protein spots were found to be with single protein identities while 2 spots, spot No. 38 & 53, were found to contain two proteins each on sequencing. These 30 identified proteins exhibited sequence homology with proteins from *Arabidopsis thaliana* (13), *Oryza sativa* (4), *Arachis hypogaea* (6), *Glycine max* (1), *Pisum sativum* (1) and *Vigna unguiculata* (1), while some proteins showed homology with cereal crops like *Zea mays* (1), *Triticum aestivum* (1) and with other crops: *Cedrelia pentastylis* (1) and *Daucus carota* (1) (Fig. 7).

Ontological classification of proteins

The 30 identified proteins were grouped into different categories based on their sub-cellular localization, biological process and molecular function according to the annotation in the Viridiplantae taxonomic database. These proteins when queried using Targetp software revealed their localization in various cell compartments, mostly plastids, cell membrane and nucleus, etc. The data on biological process showed that over 20% of proteins were defense related, 12% stress responsive, 12% signaling, 12% DNA repair, 12% carbohydrate metabolism, 12% photosynthesis, 4% detoxification and other 4% lipid biosynthesis. The molecular function of these proteins indicated their involvement in protein binding, antioxidant activity, sugar binding, kinase activity, etc. (Fig. 8). Of these, 12 proteins were sequenced using MALDI TOF-TOF (Table 4). These 12 proteins were categorized into 5 different groups; (a) molecular chaperons (LEA-1, 17.3 kDa Hsp and structural maintenance of chromosome-1), (b) signal transduction proteins (Susy-1, Calmodulin-binding protein, 14-3-3), (c) photosynthetic proteins (PS I, small and large subunits of Rubisco), (d) defense-related proteins (putative lectins and M/G binding lectins) and (e) detoxification proteins (APX-1). Corresponding spot numbers of the proteins in each variety are provided in (Supplementary Table 5).

Molecular chaperons

Among the 3 molecular chaperons, LEA-1 proteins showed over expression in 15 and 20 DS plants of ICGV 91114.

Fig. 4 The 2D gels showing the leaf proteome of peanut cultivar ICGS 76. The leaf protein extracted from drought stressed and respective control plants from 10 (a) and 20 days (b) was subjected

to 2D-gel electrophoresis as described in methods. The images were digitized and quantified densitometrically

Plants adapt to water deficit by the induction of specific genes encoding LEA proteins (Granier 1988). An increased expression of LEA proteins in ICGV 91114 might be attributed to one of the primary defense mechanisms to prevent the loss of intercellular water during drought. During water stress, LEA proteins prevent cellular collapse by adjusting osmoticum along with other hydrophilic proteins and compatible solutes (Xiong and Zhu 2002; Tunnacliffe and Wise 2007) and also act as molecular chaperones (Wise and Tunnacliffe 2004). Another class of stress inducible proteins commonly expressed during environmental stresses are small heat shock proteins (sHSPs). In our study, sHSPs with 17.3 kDa M. Wt. were up-regulated only in the 15 and 20 DS of ICGV 91114 cultivar and the other cultivars did not exhibit any expression. Similarly, Katam et al. (2016) also reported abundant expression of heat shock proteins

like disulfide isomerase-2 (PDI2) in drought-tolerant peanut cultivar, Vemana and reduced expression in susceptible cultivar. These proteins were known to assist in protein folding, assembly, translocation and degradation during plant growth and development (Park and Seo 2015). Drought stress causes unfolding and denaturation of proteins making them dysfunctional during which these sHSPs prevent the aggregation of denatured proteins and assists in refolding of non-native proteins by acting as molecular chaperones (Vierling 1991; Boston et al. 1996; Close 1996). Their function may be restored back by the interaction of 17.3 kDa HSPs with osmolytes, cell signaling molecules, cell cycling and cell death regulators (Wang et al. 2004). The other classes of proteins namely SMC-1 protein complexes have multiple functions like sister chromatid cohesion, condensation, repair of eukaryotic chromosomes and are essential

Fig. 5 The 2D gels showing the leaf proteome of peanut cultivar J 11. The leaf protein extracted from drought stressed and respective control plants from 10 (a) and 20 days (b) was subjected to 2D-gel elec-

trophoresis as described in methods. The images were digitized and quantified densitometrically

for faithful chromosome segregation (Wang et al. 2004; Lehmann 2005). Overexpression of SMC-1 protein in 15 and 20 DS plants of ICGV 91114 implies better protection of its DNA from damages leading to double-strand breaks in DNA and mutations, reduced protein synthesis, cell membrane destruction and damage to photosynthetic proteins caused by reactive oxygen species (ROS) during drought stress (Britt 1999; Nasmyth and Haering 2005).

Signal transduction proteins

The 15 and 20 DS plants of ICGV 91114 exhibited over expression of Susy-1 while all other varieties did not exhibit its expression. It is an important enzyme belonging to the family glycosyltransferase-1 that participates in carbohydrate

metabolism, catalyzing the reversible conversion of sucrose and UDP to UDP glucose and fructose in various metabolic pathways (Sebkova et al. 1995; Cooke et al. 2003). Similar results were reported in the phloem cells of leaves and roots of *Arabidopsis* (Strum et al. 1999). Phosphorylation of Susy-1, under severe drought stress, activates sucrose cleavage reaction enabling the plant to meet the increased demand for translocation of carbohydrates under limited ATP supply (Winter and Huber 2000). Calcium ion binding protein (CalM 42), also a signaling protein, was overexpressed only in the 15 and 20 DS of ICGV 91114 and did not show any expression in other 3 cultivars. In *Arabidopsis thaliana*, this protein is known to interact with calcium sensors and aid in trichome branching, the specialized epidermal structures that protect against drought stress (Dejardin et al.

Fig. 6 The 2D gels showing the leaf proteome of peanut cultivar JL24. The leaf protein extracted from drought stressed and respective control plants from 10 (a) and 20 days (b) was subjected to 2D-gel

electrophoresis as described in methods. The images were digitized and quantified densitometrically

1999; Schellmann and Hulskamp 2005; Ishida et al. 2008). The most significant class of signaling proteins, 14-3-3 proteins, were expressed more abundantly in 10, 15 and 20 DS of ICGV 91114. In ICGS 76, this protein was expressed in minute quantities compared to their controls in 10 DS and expressed more in 20 DS while it is down-regulated in 10 and 20 DS of J11. In the susceptible cultivar, JL24, the expression of this protein is almost negligible in 10 DS while 20 DS did not exhibit any expression. Katam et al. (2016) also reported the expression of these 14-3-3 proteins at higher levels in drought-tolerant peanut cultivar. These 14-3-3 proteins are a part of conserved regulatory protein family that binds to serine/threonine-phosphorylated residues expressed in all the eukaryotic cells with a striking ability to bind to signaling proteins like H⁺ ATPases, protein kinases, phosphatases and transmembrane receptors that assist in regulation of stress responsive proteins (Ferl 1996).

Binding of these 14-3-3 proteins to other phosphorylated drought stress responsive proteins might result in rapid adaptation of enzymatic activities and metabolic pathways under water stress (De Vetten and Ferl 1994).

Photosynthetic proteins

Water stress is one of the most important environmental factors inhibiting photosynthesis due to damage of chlorophyll pigments, thereby reducing their light harvesting capacity (Graan and Boyer 1990). During photosynthesis, chlorophyll pigments in photosystem-II are excited by sunlight releasing electrons and energy in the form of an ATP molecule required for the break down of water molecule (H₂O) into ½O₂ and 2H⁺ maintaining an electron gradient which is further excited in photosystem-I to produce NADP⁺⁺ H⁺. This highly energetic NADPH molecule is

Table 2 Peptide mass fingerprinting (PMF) of stress-induced proteins in ICGS 76

Sp. No.	Exptl. M.Wt.	Theo. M.Wt	Homologous protein	Organism	Biological process	Molecular function	Cellular component	Mascot score	Accession no.
4	30.5	27.2	Cytosolic ascorbate peroxidase	<i>Vigna unguiculata</i>	Hydrogen peroxide stress response	Oxidoreductase/ peroxidase activity	Cytoplasm	113	gil1420938
8	20.0	16.2	PR10 protein	<i>Arachis hypogaea</i>	Defense response/ response to biotic stimulus	–	–	76	gil52547774
15	17.0	71.4	FACT complex subunit SSRP1	<i>Zea mays</i>	DNA repair/ DNA replication/ regulation of transcription- DNA dependent	DNA binding	Chromosome/ nucleus	52	SSRP1_ MAIZE
19	21.0	16.2	PR10 protein	<i>Arachis hypogaea</i>	Defense response/ response to biotic stimulus	–	–	52	gil52547774
30	30.0	30.1	Lipoate protein ligase-like protein	<i>Arabidopsis thaliana</i>	Lipoate biosynthetic process/ Protein modification process	Octanoyl transferase activity	Chloroplast	38	gil7939551
31	27.0	22.3	Photosystem I reaction center subunit II, chloroplast precursor	<i>Arabidopsis thaliana</i>	Photosynthesis	Protein binding	Chloroplast membrane	61	PSAD2_ ARATH

Sp.No., spot number, Expl. M.Wt., experimental Mol. Wt., Theo. M.Wt., theoretical M.Wt.

then fed into the Calvin cycle to carry out carbon fixation. Hence, overexpression of Photosystem-I reaction center subunit-II of chloroplast precursor protein in the 20 DS plants of ICGV 91114, ICGS 76 and J 11 varieties might help in the uninterrupted supply of NADPH during photosynthesis to ensure the supply of substrates to carbon skeleton for various metabolic pathways conferring increased drought tolerance. However, the decline in the intracellular levels of CO₂ might create an oxidative stress leading to the destruction of photosynthetic apparatus, changes in the conformation of chloroplast proteins, imbalances in the ions and other macromolecules making JL 24 the most susceptible (Lauer and Boyer 1992; Bray et al. 2000). Ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCo) is the key photosynthetic enzyme that plays a significant role in the fixation of CO₂ in C₃ plants which can be quickly remobilized under stress (Jensen and Bahr 1977). Many studies reported the reduced expression of

RuBisCo under drought stress (Parry et al. 2002) while the studies in the drought-tolerant peanut cultivar, Vemana by Katam et al. (2016), reported high abundance of RuBisCo under stress conditions. Kottapalli et al. (2009) reported the reduction in photosynthesis-related proteins in tolerant genotypes during water stress which includes proteins like Rubisco LSU and SSU, oxygen evolving enhancer protein of PS II and chlorophyll a/b binding proteins. However, in our study, both larger and smaller subunits of Rubisco exhibited down-regulation in all the stressed plants of 4 varieties compared to their respective controls while PS I reaction centre subunit II protein showed overexpression in severely stressed plants of ICGV 91114, ICGS 76 and J11 cultivars. Overproduction of reactive oxygen species (ROS) during drought stress cleaves the large subunit of Rubisco directly or modify it to become more susceptible to proteolysis (Feller et al. 2008).

Table 3 Peptide mass fingerprinting (PMF) of water stress-induced proteins in J 11

Sp.No.	Exptl. M.Wt.	Theo. M.Wt.	Homologous protein	Organism	Biological process	Molecular function	Cellular component	Mascot score	Accession no.
8	8.64	15.6	RUBP carboxylase small chain precursor	<i>Phaseolus vulgaris</i>	Photosynthesis	Lyase/Monooxygenase	Plastid	181	gil123534
10	31.0	30.7	Alpha-methyl-mannoside-specific lectin	<i>Arachis hypogaea</i>	–	Cytokinin binding	–	283	gil15233402
11	34.0	29.2	14-3-3 protein	<i>Vigna angularis</i>	–	Protein domain specific binding	–	459	gil45594277
12	32.0	28.3	Mannose/Glucose-binding lectin precursor	<i>Arachis hypogaea</i>	–	Sugar binding	–	233	gil8099130
13	27.5	21.8	2-cys perox-iredoxinlike protein-	<i>Hyacinthus orientalis</i>	Stress response	Antioxidant activity/perox-iredoxin activity	Chloroplast/plastid	96	gil47027073
24	21.0	16.2	PR10 protein	<i>Arachis hypogaea</i>	Defense response/response to biotic stimulus	–	–	76	gil52547774
32	19.5	16.2	PR10 protein	<i>Arachis hypogaea</i>	Defense response/response to biotic stimulus	–	–	52	gil52547774

Sp.No., spot number, Exptl. M.Wt., experimental Mol. Wt., Theo. M.Wt., theoretical M.Wt.

Defense related proteins

Lectins are carbohydrate-binding proteins that specifically recognize diverse sugars and mediate a variety of biological processes (Lis and Sharon 1998). Several studies indicated the induction of different lectins with diverse functions from cell wall modification to regulation of gene expression in response to both abiotic and biotic stresses (Vijayan and Chandra 1999; Van Damme et al. 2004). In our study, alpha-methyl-mannoside-specific lectin was overexpressed only in the 20 DS plants of ICGV 91114 and either did not exhibit (or) showed almost negligible expression in other three cultivars. Similarly, the other classes of lectins like mannose/glucose-binding lectins also showed increased expression in all the stressed plants of ICGV 91114 cultivar only and showed reduced expression/negligible in other 3 cultivars. However, no expression is seen in 20 DS of JL24 cultivar. Similarly, Bhushan et al. (2007) reported expression

Fig. 7 The homology of differential peanut proteins with proteins from other crops listed in NCBI *Viridiplantae* Database

Fig. 8 Functional cataloguing of drought stress responsive proteins

of mannose lectin in stress-tolerant chickpea cultivar under water-deficit stress. In contrast, Kottapalli et al. (2009) reported overexpression of lectins like galactose-binding and mannose/glucose-binding isoforms only in the susceptible genotype and undetectable in tolerant genotypes. Lannoo and Van Damme 2014 in their extensive research on lectins reported that plants express minute amounts of specific lectins only upon environmental stresses, while most of the lectins involved in plant defense are constitutively expressed in high amounts in seeds and vegetative storage tissues.

Detoxification proteins

Cytosolic ascorbate peroxidase-1 (APX-1) enzyme that plays a key role in regulation and signaling of H_2O_2 in plant cells is yet another protein that accumulated in larger quantities in all the stressed plants of ICGV 91114, ICGS 76 and J 11 varieties under drought stress. During drought stress, ROS produced in the chloroplasts and peroxisomes traverse to the nuclei through cytosol (Davletova et al. 2005; Asada 2006; Van Breusegem and Dat 2006). APX-1 is required for the protection of chloroplasts against ROS and in its absence, the ROS scavenging machinery is significantly compromised (Mittler et al. 2004). Grimplet et al. (2009) reported that increased production of ascorbate by APX activity restored the oxidation levels during stress in the drought-tolerant cultivar. APX was significantly down-regulated in 20-day stressed plants of JL 24 leading to the accumulation of hydrogen peroxide in the cytosol triggering cell injury and death and thus making this variety the most drought susceptible. Similarly, Katam et al. (2016) reported negative interaction of APX in the drought susceptible peanut cultivar.

Comparative analysis of expression levels of identified proteins in 4 varieties

The following proteins: cytosolic ascorbate peroxidase, photosystem I reaction center subunit II of chloroplast precursor,

alpha-methyl-mannoside-specific lectin, mannose/glucose binding lectin and 14-3-3 proteins were identified and analyzed for their differential levels of expression in 10 and 20 DS plants of all the 4 varieties. There was an increase in the expression levels of APX protein by 1.5 folds indicating its up-regulation in 10 DS plants compared to their respective controls in all the 4 varieties, while in 20 DS plants of ICGV 91114, ICGS 76 and J 11 there was an increase by twofold exhibiting over expression, whereas in JL 24 this protein exhibited a significant down-regulation. Photosystem I reaction center subunit II of chloroplast precursor protein exhibited an increased expression by > 2 folds in 20 DS plants of 3 varieties while this protein did not express in rest of the treatments and controls (Fig. 9a). The alpha-methyl-mannoside-specific lectin exhibited upregulation only in the 20 DS plants of ICGV 91114 and mannose/glucose binding lectin proteins exhibited upregulation in the 10, 15 and 20 DS of ICGV 91114 while 14-3-3 proteins exhibited upregulation in 10, 15 and 20 DS of ICGV 91114 and only 20 DS of ICGS 76 (Fig. 9b).

Conclusion

Considering the prominent role peanut crop plays in our economy as an oilseed crop, identification of genotypes tolerant to drought stress plays a significant role in improving productivity levels. The 30 drought-tolerant proteins identified in the present investigation could be used as a reference library for probing the drought-tolerant proteins in other crops. The twelve proteins that were sequenced namely LEA-1, calcium ion binding protein, Susy-1, 17.3 kDa heat shock protein, SMC-1, cytosolic ascorbate peroxidase, 14-3-3 proteins, alpha-methyl-mannoside-specific lectin, mannose/glucose-binding lectins, PS I and Rubisco were reported to confer a significant role in drought stress from previous studies and also the current study. A repository of these proteins provides a basis for exploring

Table 4 MS/MS sequences of MALDI-TOF identified drought-tolerant proteins in peanut

Sp. No.	Exptl. M.Wt.	Protein identified	Accession no.	Mascot score	Sequence coverage	Matching peptide sequence in red
53	21.5/5.8	Late embryogenesis abundant protein	gil56709428	66	50	MASRQDRREA RAEADARRAA EEIARARDER VMQAEVDARS AADEIARARA DRGAATM- GAD TAHHAAGGGG ILESVQEGAK SFVS- AVGRTF GGARDTAAEK TSQTADATRD KLGEYKDYTA DKARETNDSV ARKTNETADA SRDKLGEYKD YTADKTRETK DAVAQKASDA SEATKNKLGE YKDALARKTR DAKDTTAAQKA TEFKDGVKAT AQETRDATAD TARKAKDATK DTTQTAADKA RETAATHDDA TDKGQGQGLL GALGNVTGAI KEKLTVSPAA TQEHLGGGEE RAVKERAAEK AASVYFEEKD RLTRERAAER VDKCVEKCEV GCPDATCAHR HGKM
53	21.5/5.8	Calcium ion binding	gil15233402	75	24	MESNNNEKKK VARQSSSFRL RSPSLNALRL QRIFDLFDKN GDGFITVEEL SQALTRLGLN ADLSDLKSTV ESYIQPGNTG LNFDDFSSLH KTLDDSFEGG ACGGGENEDD PSSAAENESD LAEAFKVFDE NGDGFISARE LQTVLKKLGL PEGGEMERVE KMIVSVDRNQ DGRVDFFEFK NMMRTVVIPS S
38	19.5/6.3	Sucrose synthase isoform 1	gil1351139	82	33	MGEPLVLRVH SLRERMDSTL ANHRNEILMF LSRIESHGKG ILKPHQLLAE YEAIKEDKL KLDDGHGAFA EVIKSTQEI VSPPWVALAI RLRPGVWEYV RVNVHHLVVE ELSVPQYLQF KEELVIGSSD ANFVLELDFA PFTASF- PRPT LTKSIGNVE FLNRHLSAKM FHGK- DSMHPL LEFLRLHYNV GKTMLNLRV QNVNGLQSM LKAGDYLSTL PSDTPYSEFE HKFQEIGFER GWGDTAERTV EMFHMLLDLL EAPDASTLET FLGKIPMVFN VVILSPHGYF AQENVLGYPD TGGQVVYILD QVPALEREMI KRIKEQGLDI KPRILIVTRL LPDAVGTTTCN QRLEKVFGEA HAHILRVPFR TEKGI LRKWI SRFEVWPYIE TFTEDVAKEI ALELQAKPDL IIGNYSEGNL VASLLAHKLG VTQCTIAHAL EKTYPDSDI YWEKFDKKYH FSSQFTADLI AMNHTDFIIT STFQEIAGSK DTVGQYESHT AFTMPGLYRV VHGIDVFDPK F-VSPGADT SVYFSYKEKE KRLTTLHPEI EELLYSSVEN EEHLCHKDK NKPIFTMAR LDNVKNLTGF VEWYAKSPKL RELVNLVVVG GDRRKESKDL EEQAQMKKMY ELIDTYKLNG QFRWIS- SQMN RVRNGELYRY IADTKGAFVQ PAF- YEAFLGT VVEAMTCGLP TFATLHGGPA EIVHGKSGF HIDPYHGEQV AELLVNFEEK CKTDPQWDA ISAGGLKRIQ EKYTWQIYSE RLTLAGVYG FWKHVSKLDR LEIRRYLEMF YALKYRKLAE SVPLAKDE

Table 4 (continued)

Sp. No.	Exptl. M.Wt.	Protein identified	Accession no.	Mascot score	Sequence coverage	Matching peptide sequence in red
38	19.5/6.3	Structural maintenance of chromosome 1	gil45594277	73	31	MPAIQSPSGK ILQLEMENFK SYKGHQLVGP FKDFTAIIGP NGSGKSNLMD AISFVLGVRT GQLRGSQKLD LIYAFDDRDQ EQRGRKAFVR LVYQMDDGVE LRFTR SITSA GGSEYRIDNR VVNLDEYNGK LRS LGILVKA RNFLVFQGDV ESIASKNPKE LTGLLEEISG SEELKKEYEG LEEKKASAE KAAL LYQKKK TIGNEKKLKK AQKEEA EKHL RLQEELKALK RERFLWQLYN IENDIEKANE DVDSEKSNRK DVMRELEKFE REAGKRKVEQ AKYL KEIAQR EKKIAEKSSK LGKIQPELLR FKEEIARIKA KIETNRKDVD KRKKEKGKHS KEIEQM QKSI KELNKKMELF NKKRQDSSGK LPMLDSQLQD YFRLKEEAGM KTIKLRDEHE VLERQRR TDL EALRNLEENY QQLINRKNDL DEQIKRFKDR QGEIETSSSK YKNETTSLKT ELRALQEKHV NAREASAKLK TRIAELEDQL SDLTAERYEN ERDSRLTQAV ESLK RLFQGV HGRMTDL CRP NRKKYNLAVT VAMGRFMDAV VVEDENTGKD CIKYLKEQRL PPMTFIPLQS VRVKQVFERL RNLGGTAKLV FDVIQFDP EL EKAVLYAVGN TLVCDELEEA KVL SWSGERF KVVTVDGILL TKAGTMTGGT SGGMEAKSNK WDDKKIEGLK KNKEDFEQQL ENIGSIREMQ MKESEISGKI SGLEK KIQYA EIEKKS IKDK LPQLEQEERN IIEIDRIKP EL SKARTEVD KRK TEMNKLE KRMNEIVDRI YKDFSQSVGV PNIRVYEETQ LKTAEKEAEE RLELSNQPAK LKYQLE YEQN RDVGSRIKI ESSISSLET D LEGIQKTMSE RKETAVKITN EINNWKEME ECKQK SEYE KEILDWKKQA SQATT SITKL NRQI SKETQ IEQLISQKQE ITEKCELEHI TLPVLS DAME EDDSDGPQFD FSELGRAYLQ ERRPSAREKV EAEFRQKIES KTSEIERTAP NLRALDQYEA IQEKEKQVSQ EFEAARKEEK QVADAFNTVK QKRYELFMEA FNHIASNIDK IYKQLTKSNT HPLGGTAYLN LENEDDPFLH GIKYTTMPPT KRFRDMEQLS GGEKTVAALA LLFSIHSYRP SPFFILDEV D AALDNLNVAK VAKFIRSKSC QAARDNQDAE DGNGFQSIVI SLKDSFYDKA EALVG VYRDT ERSCSSTMSF DLRNYQES
43	17.3/5.9	17.3 kDa class I heat shock protein	gil123534	70	40	MSLIPSF FGG RRSSVDFPFS LDVWDPFKDF PFPSSLSAEN SAFVSTR VDW KETPEAHVFK ADIPGLKKEE VKLEIQDGRV LQISGERNVE KEDKNDTWHR VERSSGKLV RFR LPENAKV DQVKASMENG VLT VTVPKEE IKKPDVKAID ISG
4	30.5/6.1	Cytosolic ascorbate peroxidase	gil1420938	113	20	MGKSYPTVSP DYQKAIEKAK RKL RGFIAEK KCAPLILRLA WHSAGTFDSK TKTGGPFGTI KHQAELAHGA NNGLDIAVRL LEPIKEQFPV- SYADFYQLA GVVAVEITGG PEVPFHPGRE DKPEPPPEGR LPDATKGS DH LRDVFGKAMG LSDQD IALS GGHTIGAAHK ERSGFEGPWT SNPLIFDNSY FTELLTGEKD GLLQLPSDKA LLTDSVFRPL VEKYAAEDDV FFADYAE AHL KLSELGFAEA

Table 4 (continued)

Sp. No.	Exptl. M.Wt.	Protein identified	Accession no.	Mascot score	Sequence coverage	Matching peptide sequence in red
31	27/6.3	Photosystem I reaction center subunit II, chloroplast precursor	PSAD2_ARATH	61	9	MATQAAGIFN SAITTAATSG VKKLHFFSTT HRPKSLSTFK TAIRAECTDS SAAAAAAPAT KEAPVGFTTP QLDPNTSPFI FAGSTGGLLR KAQVEEFYVI TWNSPKEQIF EMPTGGAAIM REGPNLLKLA RKEQCLALGT RLR SKYKITY QFYRVFPNGE VQYLHPKDG VYPEKANPGRE GVGLNMRSIG KNVSPIEVK FTGKQSYDL
10	31/4.5	Alpha-methyl-mannoside-specific lectin	gil15233402	283	50	MAISKKILPL LSIATIFLLL LNK AHSLGSL SFGYNNFEQG DERNLILQGD ATF SASKGIQ LTKVDDNGTP AKSTVGRVLH STQVRLWEKS TNRLTNFQAQ FSFVINSPID NGADGIAFFI AAPDSEIPKN SAGGTLGLSD PSTAQNP PSAN QVLAVEFDTF YAQDSNGWDP NYQHIGFDVD PIK SAATKW ERRNQTLNV LVS YDANSKN LQVTASYPDG QSYQVSYNVD LRDY LPEWGR VGFSAA SGQQ YQSHGLQSW FTSTLLYTSP HYLKLGRFMI
11	34/4.6	14-3-3 protein	gil45594277	459	50	MAAAPTPREE NVYMAKLAEQ AERYEEMVEF MEKVSAAADN EELNVEERNL LSVAYKNVIG ARRASWRIIS SIEQKEESRG NEDHVTVIRD YRSKIESELS NICDGILKLL DSRLIPSASS GDSKVFFYLMK KGDYHRYLAE FKTGAERKEA AESTLAAYKS AQDIANAELP PTHPIRLGLA LNFVVFYIEI LNSPD RACNL AKQAFDEAIA ELDTLGEESY KDSTLIMQLL RDNLTLWTSD MQDDGADEIK EAAPKQDDQ
12	32/4.7	Mannose/glucose-binding lectin precursor	gil8099130	233	50	LDSLSFSYNN FEQDDERNLI LQGD AKFSAS KGIQLTKVDD NGTPAKSTVG RVLHSTQVRL WEKSTNRLTN FQAQFSFVIK SPIDNGADGI AFFIAAPDSE IPKNSAGGTL GLFDPQTAQN PSANQVLAVE FDTFYAQDSN GWDPNYQHIG IDVNSIKSAA TKWERRDGQ TLNVLVTYDA NSKNLQVTAS YPDGQRYQLS YRVDLRDYLP EWGRVGFSA SQQQYQSHEL QSWSTSTLL YTSPLYLKLGRFMI
1	56/6.2	RUBP carboxylase large chain	gil1351139	519	50	MSPQTETKAS VGFKAGVKDY KLTYYTPEYE TKDTDILAAF RVTPQPGVPP EEAGAAVA AE SSTGTWTTVW TDGLTSLDRY KGRCYHIEPV AGEENQYIAY VAYPLDLFEE GSVTNMFTSI VGNVFGFKAL RALRLEDLRI PTSYSKTFQG PPHGIQVERD KLNKYGRPLL GCTIKPKLGL SAKNYGRAVY ECLRGGLDFT KDDENVNSQP FMRWRDRFLF CAEALFKAQA ETGEIKGHYL NATEGTCEEM IKRAVFAREL GAPIVMHDYL TGGFTANTSL AHYCRDNGLL LHIH RAMHAV IDRQKNHGMH FRVLAKGLRL SGGDHIHAGT VVGKLEGERD ITLGFVDLLR DDFIEKDRSR GIYFTQDWVS LPGVLPVASG GIHVWHMPAL TEIFGDDSVL QFGGGTLGHP WGNAPGAVAN RVALEACVQA RNEGRDLARE GNEI IREASK WSPELAAACE VWKEIKFEFE AMDTL
8	8.64	RUBP carboxylase small chain precursor	gil123534	181	50	TSVANNGGRV QCIQVWPTVG KKKFETLSYL PPLTKQQLAK EVDYLLRKGW VPCLEFELEH GFVYREHNKS PGYYDGRYWT MWKLPMF- GCT DSSQVLKELY EAQTAHPDGF IRIIGFD- NVR QVQCISFIAY KPPGY

Peptides of *Arachis* proteins sequenced through MS/MS are shown in bold which are matching with the peptides of Viridiplantae taxonomic group

Fig. 9 a Comparison of 2DE gels (4–7 pH) exhibiting the differential expression of cytoplasmic APX and PSI proteins in 4 varieties of groundnut. **b** Comparison of 2DE gels (4–7 pH) exhibiting the differential expression of putative lectins in 4 varieties of groundnut

A ICGV 91114

ICGS 76

J 11

JL 24

B ICGV 91114

ICGS 76

J 11

JL 24

possible protein–protein interactions and for further functional genomic studies. These proteins could be exploited as protein markers to evaluate the peanut germplasm and other crop plants for drought tolerance.

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Author contribution PT, MRD and GBR conceived of this study and designed the experiments. PT isolated proteins from samples, done all proteomics experiments and analyzed the data and PNBS supported experiments. PT and MRD drafted the manuscript and GBR critically evaluated and proof-read the manuscript. RP supported manuscript corrections.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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