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Physiological and proteomic analysis of two contrasting *Sorghum bicolor* genotypes in response to drought stress

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Abstract

Understanding the response of a crop to drought is the first step in the breeding of tolerant genotypes. In this study, two sorghum (*Sorghum bicolor* L.) genotypes with contrasting sensitivity to drought were subjected drought stress by withholding water for 7 days at seedling stage; physiological and protein analyses were made. Reduction percentage was recorded on leaf water content, chlorophyll a and b and total chlorophyll. Shoot and root lengths reductions were observed in the drought-sensitive Cultivar (Tabat) while the drought-tolerant line (EL9) showed an increase in shoot and root lengths under drought conditions. Drought tolerant sorghum line EL9 accumulated higher proline (26% increase) when compared to the sensitive cultivar Tabat (5% increase). Mass spectrometry analysis coupled with nanoflow UPLC was used to compare daily-watered with drought stressed (7 days) seedlings. A total of 36 protein spots were detected, of which 23 were recorded for one or both accessions under drought stress conditions only. These proteins were identified using MASCOT database search in accordance with sequence similarity with previously characterized proteins from the Uniprot database. The identified proteins were assigned to different functional categories as follows: Response to stress (35%); metabolic processes (26%); photosynthetic (13%); fatty acid biosynthesis (4%) and cell wall biogenesis/degradation (4%). Seven of the identified proteins under stress condition were unique to El9, in contrast to 4 proteins were unique to Tabat. This study showed a differential protein expression pattern of two sorghum accessions under drought stress, which will be valuable for studying the molecular mechanisms underlying drought tolerance in the future. Also, these proteins could be potential candidates for development of markers to be used in markers assisted selection.

Keywords: sorghum, drought stress, proteomic, mass spectrometry.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important crop in the world which is used as food, feed, fodder, fiber, and fuel. Sorghum is better adapted to marginal, hot, and drought prone environments compared with most other crops. This attribute is of great importance as the demand for food and water supplies increases due to world population growth (Ejeta and Knoll, 2007).

Abiotic and biotic stresses limit plant growth and crop productivity. Changes in precipitation patterns due to climate change and meteo- climatic variability have become a critical issue and a limiting factor for the crops under rain-fed systems. As water is sometimes limiting under irrigation systems due to competition between crops, drought tolerant genotypes are also preferred (Anami et al., 2015).

Plant drought tolerance is a complex quantitative trait, involving multiple metabolic pathways. A number of genes involved in plant drought responses and tolerance have been identified in model plants and crops (Seki et al., 2002; Guo et al., 2009; Campo et al., 2012). Knowledge concerning the molecular mechanisms underlying drought tolerance is one of the critical factors needed for the development of drought tolerant genotypes. Due to the large distribution of semi-arid agricultural lands in Sudan, species with higher water-use efficiency (WUE) and drought tolerance are required for production (Somerville et al., 2010).

Proteomics is the large-scale analysis of protein from a particular organism has been used to study global changes in the protein expression in plant tissues, cells and sub-cellular compartments (Blackstock and Weir, 1999; van Wijk, 2001). Drought induces the expression of proteins that are not specifically related to water deficit, but which are induced by cellular damage. These include different classes of heat shock protein genes or cognates (Kiyosue et al., 1994), thiolproteases (Williams et al., 1994), proteinase inhibitors (Reviron et al., 1992), and osmotin (Kononowiczet al., 1993).

Protein expression changes in response to drought have been reported in rice (Ali and Komatsu, 2007), maize (Riccardi et al., 1998), sugar beet (Hajheidari et al., 2005), wheat (Hajheidari et al., 2007) and sunflower (Castillejo et al., 2008). A number of drought-induced proteins which are involved in photosynthesis, signaling pathways, oxidative stress detoxification were identified in different crops (Ali and Komatsu, 2007).

The aim of this study was to investigate the influence of drought stress on the physiological and biochemical parameters of one drought tolerant line (El9, recently developed and purified) and a drought sensitive sorghum

cultivar (Tabat). Also, to explore and identify stress responsive proteins following drought treatment using mass spectrometry analysis couple with nanoflow Ultra Performance Liquid Chromatography (UPLC).

Results and Discussion

Physiological response to drought stress

The two sorghum accessions revealed significant differences ($P < 0.05$) under drought stress for all measured characters (Table 1).

Reduction in both shoot and root lengths were observed for Tabat cultivar while EL9 showed an increase in both measured parameters (Table 2). High reduction percentage (47%) was recorded on leaf water content for the sensitive sorghum cultivar (Tabat) when compared to the drought tolerant line (EL9) for which 5.5% reduction was recorded (Table1). Low LWC indicates a negative plant response to drought stress whereas high leaf water content prevents leaf wilting under stress (Liu et al., 2002). The reduction in shoot and root growth is an important drought-induced stress (El Midaoui et al., 2003). According to the obtained data, El9 was found to retain higher leaf water content and higher shoot and root growth, respectively, under stress conditions compared to Tabat, this results is in accordance with Maheswari et al. (2010). Bibi et al. (2010) observed that most of the morphological and physiological characters at seedling stage were affected by water stress in sorghum. Drought stress suppressed shoot and root growth and in certain cases root growth increased (Salih et al., 1999; Bibi et al., 2010). Similar trends of variable response are also reported by Achakzai and Bazai (2007) in some sorghum cultivars. Percent reduction in chlorophyll a and total chlorophyll contents recorded for Tabat in response to drought were significantly ($P>0.05$) higher than those recorded for El9 (Table 1 & 2). However, reduction % in chlorophyll b was significantly higher for El9 compared to Tabat (Table 1&2). Similarly, Ommen et al. (1999) reported that total leaf chlorophyll content decreases as a result of drought stress. Drought stress caused a large decline in the content of chlorophyll a, chlorophyll b, and total chlorophyll in all sunflower varieties investigated by Manivannan et al. (2007). Severe drought stress inhibits photosynthesis in plants by: causing changes in chlorophyll content, affecting chlorophyll components and damaging the photosynthetic apparatus (Iturbe-Ormaetxe et al., 1998). The results are in agreement with Nyachiro et al. (2001), who reported a significant decrease in chlorophyll *a* and *b* due to water deficit in six *Triticum aestivum* cultivars.

Decrease in osmotic potential due to stress results in the increased hydrolysis of macromolecules into simpler ones like simple sugars and amino acids resulting in higher solute concentration (Tyagi et al., 1999). In line with this, significantly higher increase (26%) in proline concentration was detected in stressed El9 seedlings. It should be emphasized that Tabat accumulated less proline (5%) when compared to El9 (Table 1&2). This result which indicates a better performance of El9 under water stress conditions compared to Tabat. Johnson et al. (2015) have also reported the accumulation of proline during drought stress. Proline acts as an osmoticum and accounts for higher drought tolerance due to greater relative water content and leaf water (Ashraf and Foolad, 2007). Thus, proline accumulation is believed to play adaptive roles in plant stress tolerance (Verbruggen and Hermans, 2008) and has been advocated as a parameter of selection for stress tolerance (Yancy et al., 1982; Jaleel et al., 2007).

Proteomic analysis

Proteomic profiling was used to monitor changes in response to drought conditions in the tolerant line (El9) and a drought sensitive sorghum cultivar (Tabat). Differential expression between stressed and non-stressed seedlings was assessed using mass spectrometry coupled with the MASCOT database search in accordance with sequence similarity for previously characterized proteins from the Uniprot database. Comparison of the differential protein expression between the two accessions was also considered. The extracted proteins were subjected to trypsin digestion and analyzed on a high resolution mass spectrometry analysis couple with nanoflow UPLC. The detailed protein and peptide identification information for drought tolerant line (El9) and sensitive cultivar (Tabat) were listed in Tables 3 and 4, respectively. Twenty three proteins were detected under drought stress for the two accessions. The identified proteins were assigned to different functional categories (Table 3, 4 and Fig. 2) as follows: Response to stress (35%); metabolic processes (26%); photosynthetic (13%); fatty acid biosynthesis (4%) and cell wall biogenesis/degradation (4%). Seven of the identified proteins under stress condition were unique to El9, In contrast, to 4 proteins were unique to Tabat. Drought induced proteins identified only on the drought tolerant line (El9), are suggested to play important roles in sorghum drought tolerance. Drought stress can result in changes in the protein content through changes in gene expression or altered protein stability, degradation or modifications accompanying various cellular processes that reflect drought-induced damage/metabolism failure and adjustment, adaptation and homeostasis maintenance. The majority of the proteins identified in this study responded to drought stress in both compared genotypes. This finding indicates that the differential sensitivity of the examined genotypes to drought is associated with changes in a limited fraction of proteins and/or depends on the extent of the quantitative changes in protein levels. Similar results were observed by Peng et al. *(*2009) who found cultivar-specific differences in the drought/salinity-induced changes (37% and 9% of differentially expressed proteins for the root and leaf proteomes, respectively) of the wheat proteome; many of these differences involved antioxidant protein.The most represented functional category of proteins responding to drought in our case contained various chaperones, heat-shock proteins and other proteins that participate in protein folding. These proteins were also among those that showed the strongest response to stress conditions. Similarly, Xu and Huang (2010) reported an increase in the abundance of several HSPs in a drought-tolerant cultivar of Kentucky bluegrass but not in a drought-sensitive cultivar. Genotype-dependent changes in HSP were observed in the leaves of eight poplar genotypes subjected to an insufficient water supply (Bonhomme et al., 2009). Veeranagamallaiah et al. (2011) have also suggested that LEA proteins could act as a special form of molecular chaperones that would prevent the aggregation of otherVeeranagamallaiah *et al.* (2011) have also suggested that LEA proteins could act as a special form of molecular chaperones that would prevent the aggregation of other proteins induced by water stress.

Table 1. Analysis of variance of the two sorghum accessions under drought stress.

Source	***SS***	***df***	***MS***	
Between-treatments	61.9663	1	61.9663	F = 6.75646
Within-treatments	275.1424	30	9.1714	
Total	337.1086	31		

* The f-ratio value is 6.75646. The p-value is 0.01435. The result is significant at $p < 0.05$.

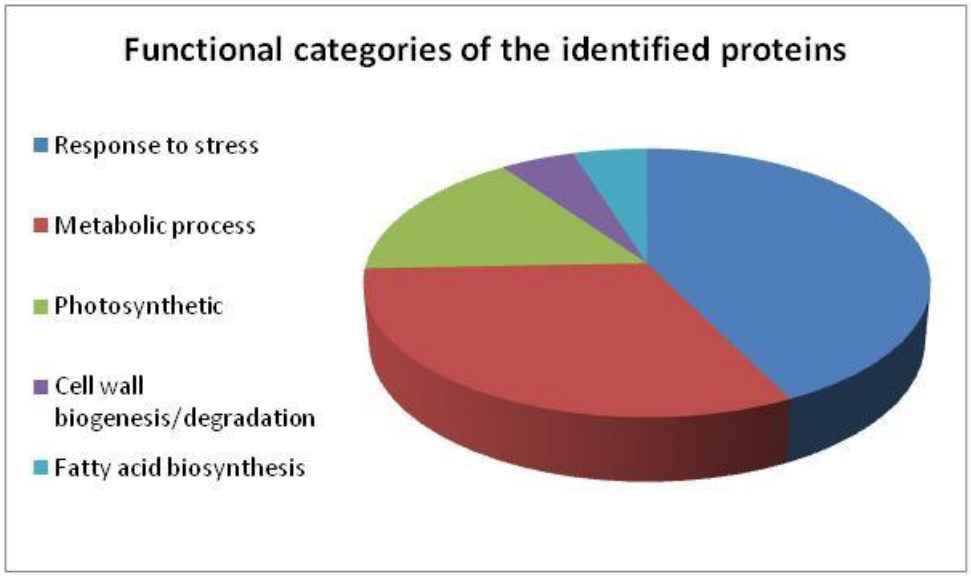

Fig 1. Pie charts of different protein functional categories.

Table 2. Effect of drought stress on some parameters of sorghum Accessions.

Parameter	Sorghum accessions	Control	Drought	Reduction /Increase %
Chlorophyll a (mg/g ^{FW})	Tabat	0.23	0.16	- 30.4
	El9	0.17	0.17	0
Chlorophyll b (mg/g ^{FW})	Tabat	0.14	0.13	-7.1
	El9	0.20	0.17	- 15
Chlorophyll content (mg/g ^{FW})	Tabat	0.37	0.30	- 18.9
	El9	0.36	0.34	- 5.5
Chlorophyll a:b ratio	Tabat	1.64	1.30	- 20.7
	El9	0.85	1.00	+17.6 *
LWC (g/g^{-1})	Tabat	0.17	0.09	- 47
	El9	0.18	0.17	- 5.5
Shoot Length (cm)	Tabat	30.5	29.9	- 1.9
	El9	22.1	25.6	+15.8 *
Root length (cm)	Tabat	3.5	2.5	28.5
	El9	3.9	4.8	+23 *
Proline (mg/100g)	Tabat	662.5	698.5	+5*
	El9	579.1	730.1	+26.1*

*+ Increase; - = Reduction

Table 3. Proteins identified under drought stress in El9.

	Accession	Protein name/species	Score	Coverage	# Proteins	# Unique Peptides	# PSMs	MW [kDa]	calc. pI	Biological process
1	C5Y2U3	Acyl carrier protein OS=Sorghum bicolor	0.00	8.07	1	1	4	15.5	5.36	Fatty acid bio synthesis
2	A1E9T2	Ribulose bisphosphate carboxylase large chain OS=Sorghum bicolor	1.62	2.31	1	1	1	52.7	6.80	Photosynthesis
3	C5XXB8	GrpE protein homolog OS=Sorghum bicolor	0.00	4.18	1	1	1	37.0	4.61	Plant stress
4	A1E9T7	Apocytochrome f OS chaperon=Sorghum bicolor	9.11	7.50	76	2	5	35.4	9.03	Photosynthesis
5	P17606	Malate dehydrogenase [NADP] 1, chloroplastic OS=Sorghum bicolor	8.92	3.50	1	1	3	46.4	6.10	Metabolism
6	Q9ATM6	Aquaporin PIP2-4 OS=Zea mays	1.8	4.53	1	1	2	30.9	9.04	Plant stress
7	A2YWQ1	Heat shock protein 81-1 OS=Oryza sativa subsp. Indica	3.99	2.43	4	2	2	80.1	5.07	Plant stress
8	O49344	Putative oxygen-evolving enhancer protein 2-2 OS=Arabidopsis thaliana	3.81	7.20	13	1	2	13.4	6.07	Photosynthesis
9	Q5Z974	ATP-dependent zinc metalloprotease FTSH 1, chloroplastic OS=Oryza sativa subsp. Japonica	2.93	2.92	1	1	1	72.7	5.69	Protein metabolism/ proteolysis
10	Q7F9I1	Chaperone protein ClpC1, chloroplastic OS=Oryza sativa subsp. Japonica	0.00	1.53	2	1	1	101.7	6.51	Plant stress
11	P84977	Glycine-rich RNA-binding protein 3 (Fragments) OS= Arabidopsis thaliana	1.95	20.83	4	1	1	5.2	4.53	Plant stress
12	C5YC80	Cysteine synthase OS=Sorghum bicolor	0.00	2.80	1	1	3	41.6	8.40	Metabolism
13	Q40677	Fructose-bisphosphate aldolase, chloroplastic OS=Oryza sativa subsp. Japonica	18.04	5.41	1	2	9	42.5	7.10	Metabolism

Table 4. Proteins identified under drought stress in Tabat.

	Accession	Protein name/species	Score	Coverage	# Proteins	# Unique Peptides	# PSMs	MW [kDa]	calc. pI	Biological process
1	C5Y2U3	Acyl carrier protein OS=Sorghum bicolor	5.52	9.09	1	1	4	15.1	5.31	Metabolism
2	C5XJZ0	40S ribosomal protein S4 OS=Sorghum bicolor	0.00	3.77	1	1	1	29.9	10.15	Translation/stress
3	Q9ATM6	Aquaporin PIP2-4 OS=Zea mays	4.66	5.21	1	1	2	30.3	7.01	Plant stress
4	Q9STW6	Heat shock 70 kDa protein 6, chloroplastic OS=Arabidopsis thaliana	1.92	1.25	1	2	2	76.5	5.20	Plant stress
5	A1E9T1	ATP synthase subunit beta, chloroplastic OS=Sorghum bicolor	0.00	1.81	1	1	1	54.0	5.43	Protein metabolism/ proteolysis
6	Q7F9I1	Chaperone protein, chloroplastic OS=Oryza sativa subsp. Japonica	2.50	1.53	2	1	1	101.7	6.51	Plant stress

7	O65101	Photosystem I reaction center subunit VI, chloroplastic OS=Zea mays	2.18	7.75	1	1	1	14.9	10.10	Photosynthesis
8	P80607	Alpha-1,4-glucan-protein synthase [UDP-forming] OS=Zea mays	1.98	1.92	1	1	2	41.2	6.13	Cell wall biogenesis/degradation
9	C5YC80	Cysteine synthase OS=Sorghum bicolor GN=Sb06g001610	1.67	2.85	1	1	3	41.3	8.43	Metabolism
10	Q40677	Fructose-bisphosphate aldolase, chloroplastic OS=Oryza sativa subsp. Japonica	18.04	5.41	1	2	9	42.0	6.80	Metabolism

Table 5. Differential protein pattern for the two sorghum accessions.

S. no.	Protein name/Species	El9	Tabat
1	Acyl carrier protein OS=Sorghum bicolor	+	+
2	Ribulose bisphosphate carboxylase large chain OS=Sorghum bicolor	+	-
3	GrpE protein homolog OS=Sorghum bicolor	+	-
4	Apocytochrome f OS chaperon=Sorghum bicolor	+	-
5	Malate dehydrogenase [NADP] 1, chloroplastic OS=Sorghum bicolor	+	-
6	Aquaporin PIP2-4 OS=Zea mays	+	+
7	Heat shock protein 81-1 OS=Oryza sativa subsp. Indica	+	-
8	Heat shock 70 kDa protein 6, chloroplastic OS=Arabidopsis thaliana	-	+
9	Putative oxygen-evolving enhancer protein 2-2 OS=Arabidopsis thaliana	+	-
10	ATP-dependent zinc metalloprotease FTSH 1, chloroplastic OS=Oryza sativa subsp. Japonica	+	+
11	Chaperone protein ClpC1, chloroplastic OS=Oryza sativa subsp. Japonica	+	+
12	Glycine-rich RNA-binding protein 3 (Fragments) OS= Arabidopsis thaliana	+	-
13	Cysteine synthase OS=Sorghum bicolor	+	+
14	40S ribosomal protein S4 OS=Sorghum bicolor GN=Sb03g014380	-	+
15	Photosystem I reaction center subunit VI, chloroplastic OS=Zea mays	-	+
16	Alpha-1,4-glucan-protein synthase [UDP-forming] OS=Zea mays	-	+
17	Cysteine synthase OS=Sorghum bicolor	+	+
18	Fructose-bisphosphate aldolase, chloroplastic OS=Oryza sativa subsp. Japonica	+	+

Present(+)/Absent(-)

Stress-related proteins

These proteins have direct relation to drought and include: 40S, 60S ribosomal proteins, GrpE protein homolog, Aquaporin, Heat shock proteins, Chaperone protein and Glycine-rich RNA-binding protein. Heat shock proteins, Chaperone, aquaporins and 40S ribosomal proteins were identified on both accessions, while GrpE protein homolog, Glycine-rich RNA-binding protein were identified only on the drought tolerant line (El9) (Table 3, 4, 5). The molecular chaperons were highly expressed in plant under drought stress. They are involved in protecting macromolecules such as enzymes and lipids under severe drought stress (Zhu et al., 1997). Identified ribosomal proteins are incorporated in the cytosolic ribosome under specific situations, such as certain developmental stages, tissues, and stress conditions (Byrne, 2009). Aquaporins were identified as stress responsive proteins and are shown to be expressed in visibility of cell enlargement and cell elongation (Ingela et al., 2000).

The heat shock proteins (Hsp) are molecular chaperones involved in a variety of cellular processes including protein folding, protein transport across membranes, modulation of protein activity, regulation of protein degradation, and prevention of irreversible protein aggregation under stress conditions (Wang et al., 2004). In this study two HSPs were

identified on sorghum under drought. HSPs are regulated by the GrpE protein, a stress induced protein, was only identified in El9. This protein is known to accelerate the activity of Hsp by inducing the release of an energy substrate (Mally and Witt, 2001). Kim et al. (2015) identified two pathogenesis-related proteins, an abscisic stress-ripening protein and heat shock protein 1, expressed only under drought conditions.

Glycine-rich RNA-binding protein, a drought responsive protein, was detected on El9 and is known to play a role in RNA transcription or processing during stress; it is involved in the regulation of abscisic acid and stress responses (Joo et al., 2010).

Metabolism-related proteins

Numerous metabolic and physiological functions are compromised when the plants are subjected to drought stress, yet the rapidly upregulated activities of protective proteins provide the first line of defense to offset some of the adverse effects (Yin et al., 2014). A number of enzymes related to energy metabolism were induced by drought stress in the sorghum accessions under study (Table 3, 4, 5). Fructose-bisphosphate aldolase, cysteine synthase and ATP-dependent zinc metalloprotease were detected in both accessions, while malate dehydrogenase was detected for El9 only. Isoforms of fructose-bisphosphate aldolase which are important metabolic enzymes in the glycolysis /gluconeogenesis path ways, and malate dehydrogenase, which are involved in carbohydrate synthesis, were identified in this study as drought-induced proteins (Table 4). Donnelly et al. (2005) reported that; 40% of the identified proteins under stress were involved in energy, primary or secondary metablolism. Similarly, Kim et al. (2015) reported that; 34% of the identified proteins under drought stress were involved in metabolism. Ahsan et al. (2007) also identified proteins related to energy metabolism under water logging in tomato leaves.

Photosynthesis-related proteins

Four of the proteins identified under drought stress were classified as photosynthesis related proteins (Table 3, 4). Three of these proteins (Ribulose bisphosphate carboxylase, Apocytochrome and Putative oxygen-evolving enhancer protein) were detected on El9 while only one (Photosystem I reaction center subunit VI) for Tabat. However, Apocytochrome and Putative oxygen-evolving enhancer protein were highly found to be up regulated compared to other identified proteins (Table 2). RuBisco is involved in carbon fixation, during hot dry conditions (Whitney and Andrews, 2001). The photosystem II reaction center D1 protein is known to turn over frequently. This protein is prone to irreversible damage caused by reactive oxygen species that are formed in the light; the damaged, nonfunctional D1 protein is degraded and replaced by a new copy (Lindah et al., 2000). However, the proteases responsible for D1 protein degradation remain unknown. The possible role of the Pyrophosphate-energized vacuolar membrane proton pump, an ATP-dependent zinc metalloprotease, during this process, was investigated (Lindah et al., 2000). Oxygen evolving protein 1 identified in EL9 seedlings subjected to drought stress is known to stabilize the cluster involved in water splitting during photosystem II. This protein was also identified by Ngara et al. (2012) on sorghum seedlings raised under salinity stress. Ford et al. (2011) reported that 14% of the identified proteins under stress are involved in photosynthetic machinery.

Cell wall degradation proteins

Alpha-1, 4-glucan-protein synthase was detected for Tabat under drought stress; this protein has been reported to have a possible role in the synthesis of cell wall polysaccharides in response to salt stress (Komatsu et al., 2014).

Fatty acid biosynthesis

Acyl carrier protein, of *Sorghum bicolor* was also identified as stress responsive protein in the two sorghum accessions, which is known to play an essential cofactor role in the synthesis and subsequent desaturation and acyl transfer of fatty acids in plants and bacteria (Stumpf, 1984).

Materials and methods

Plant materials

Seeds of the drought tolerant sorghum line (EL9) and those of an elite farmer preferred sensitive sorghum cultivar (Tabat) (Lux et al., 2002) were provided by Dr. Abdelwahab Hassan, Department of Agronomy, Faculty of Agriculture, University of Khartoum.

Water stress

To assess the ability of the two sorghum accessions to tolerate drought, seeds were raised in sand clay soil contained in earthen pots and watered daily for two weeks. Drought treatment was imposed on 14-day-old plants by withholding water for 7 days at seedling stage. The control plants were watered daily during the whole period. Leaf samples from both accessions were taken from the control and stressed plants. All analyses were performed on the first leaf, which was fully expanded at the beginning of the treatment (Demirevska et al., 2008).

Physiological data

Leaf water content (LWC), chlorophyll a & b contents, total chlorophyll content, chlorophyll a:b ratio, shoot length, root length and proline content were determined. LWC was measured according to the following equation: LWC (g/g)=(FW−DW)/DW, where FW is the leaf fresh weight and DW is the dry weight (Demmig and Bjorkman, 1987). Chlorophyll content was determined in 80% acetone extract. After centrifugation (20000 *g*, 20 min) the absorbance was read spectrophotometrically at 663 and 645 nm using spectrophotomerter. Total chlorophyll as well as chlorophyll *a* and *b* concentrations were calculated according to Arnon (1949). Proline content was determined using amino acid analyzer (Sykem).

All parameters were measured for seedlings under both control and drought-stress conditions in three replicates,

ANOVA analysis was done and then the results of were subjected to LSD test for significance of differences.

Proteomics analysis

Protein extraction

The protein disulfide bonds of the samples were reduced for 40 min with 5 mM dithiothreitol at room temperature and alkylated for 40 min with 15 mM iodoacetamide in the dark. The alkylated protein samples (about 100 µg) were digested overnight at 37°C with trypsin in a 1:50 enzyme-to-substrate ratio (Promega, V5113). Following digestion, the peptide mixtures were acidified with trifluoroacetic acid (TFA) to 1%, and desalted. The dried peptides were immediately subjected to nano LC-MS/MS analysis.

Data analysis

The raw data were analyzed and searched against Uniprot Sorghum bicolor protein sequence database using Proteome Discoverer 1.2 (ThermoFisher Scientific). The parameters were set as follows: the protein modifications were carbamidomethylation (C) (fixed), oxidation (M) (variable); the enzyme specificity was set to trypsin; the maximum missed cleavages were set to 2; the precursor ion mass tolerance was set to 10 ppm, and MS/MS tolerance was 0.6 Da. Only peptides that were identified with high confident were chosen for downstream protein identification analysis.

Conclusion

The proteins that were identified only in line El9 may have an important role in sorghum drought tolerance and could be a potential source of their respective genes for sorghum breeding programs aiming at developing drought tolerant cultivars. Further investigations of these proteins may also help elucidating the mechanism of drought tolerance in sorghum.

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References

Achakzai AKK and Bazai ZA (2007) Effect of water stress on seedling growth of maize cultivars: In mannitol plus culture solution. Int J Biol Biotechnol. 4(1): 37-42.

Ahsan N, Lee DG, Lee SH, Kang KY, Bahk JD, Choi MS, Lee BH (2007) A comparative proteomic analysis of tomato leaves in response to water logging stress. Physiologia Planta. 131(4): 555–570.

Ali GM and Komatsu S (2006) Proteomic analysis of rice leaf sheath during drought stress. J Proteome Res. 5:396-403.

Almeida C, Silva TD, Malafaia CB, do Amaral DV, de Souza Arruda IR, Brito GG, Donato S, Márcia VS and Maria TS (2013) Proteomic and physiological analysis of response to water deficit in sugarcane. Wudpecker Journal of Agricultural Research. 2(1): 001 – 007.

Anami SE, Zhang L, Xia Y, Zhang Y, Liu Z and Jing H (2015) Sweet sorghum ideotypes: genetic improvement of stress tolerance. Food Energy Secur. 4(1): 3-24.

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. Plant Physiol. 24: 1–15.

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Experiment Bot. 59: 206–216.

Bibi A, Sadaqat HA, Akram HM and Mohammed MI (2010) Physiological markers for screening sorghum (*Sorghum bicolour L.*). germplasm under water stress condition. Intl J Agric Biol. 12(3): 451-455.

Blackstock WP and Weir MP (1999) Proteomics: quantitative and physical mapping of cellular proteins. Trends Biotechnol. 17: 121-127.

Bonhomme L, Monclus R, Vincent D, Carpin S, Lomenech AM, et al. (2009) Leaf proteome analysis of eight *Populus xeuramericana* genotypes: Genetic variation in drought response and in water-use efficiency involves photosynthesis-related proteins. Proteomics. 9: 4121–4142.

Byrne ME (2009) A role for the ribosome in development. Trends Plant Sci. 14: 512–519.

Campo S, Peris-Peris C, Montesinos L, Peas G, Messeguer J, San Segundo B (2012) Expression of the maize *ZmGF14-6* gene in rice confers tolerance to drought stress while enhancing susceptibility to pathogen infection. J Exp Bot. 63:983–999.

Castillejo MA, Maldonado AM, Ogueta S, Jorrin JV (2008) Proteomic analysis of responses to drought stress in sunflower (*Helianthus annuus*) leaves by 2DE gel electrophoresis and mass spectrometry. Open Proteomics Journal, 1:59–71.

Demirevska K, Simova-Stoilova L, Vassileva V, Vaseva I, Grigorova B and Feller U (2008) Drought-induced leaf protein alterations in sensitive and tolerant wheat varieties. Plant Physiol. 34 (1-2):79-102

Demmigm B and Björkman O (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O2 evolution in leaves of higher plants. Planta. 171:171–184

Donnelly BE, Madden RD, Ayoubi P, Porter DR, Dillwith JW (2005) The wheat (*Triticum aestivum* L.) leaf proteome. Proteomics. 5(6): 1624–1633.

Ejeta G and Knoll JE (2007) Marker-assisted selection in sorghum In: Varshney, R.K. and R. Tuberosa (ed.) Genomic-assisted crop improvement: 2: Genomics applications in crops. Pp.187-205.

El Midaoui M, Serieys H and Kaan F (2003) Effects of osmotic and water stresses on root and shoot morphology and seed yield in sunflower (*Helianthus annuus* L.) genotypes breed for morocco or issued from introgression with *H. argophyllus* T. & G. and *H. debilis* Nutt. Helia. 26(38): 1-16.

Ford KL, Cassin A, Bacic A (2011) Quantitative proteomic analysis of wheat cultivars with differing drought stress tolerance. Front Plant Sci. 2:44.

Guo P, Baum M, Grando S, Ceccarelli S, Bai G, Li R, von Korff M, Varshney RK, Graner A, Valkoun J (2009) Differentially expressed genes between drought-tolerant and drought-sensitive barley genotypes in response to drought stress during the reproductive stage. J Exp Bot. 60:3531–3544.

Hajheidari M, Abdollahian-Noghabi M, Askari H, Heidari M, Sadeghian SY, Ober ES, Salekdeh GH (2005) Proteome

analysis of sugar beet leaves under drought stress. Proteomics. 5:950–960.

Hajheidari M, Eivazi A, Buchanan BB, Wong JH, Majidi I, Salekdeh GH (2007) Proteomics uncovers a role for redox in drought tolerance in wheat. J Proteome Res. 6:1451–1460.

Ingela J, Maria K, Urban J, Christer L, Per K (2000) The role of aquaporins in cellular and whole plant water balance. Biochem Biophys Acta. 1465: 324–342

IturbeOrmaetxe I, Escuredo PR, Arrese-Igor C, Becana M (1998) Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol. 116: 173–181.

Jaleel CA, Gopi R, Sankar B, Manivannan P, Kishorekumar A, Sridharan R, Panneerselvam R (2007) Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South Afr J Bot. 73: 190–195.

Johnson S, Cummins I, Lim FL, Slabas AR, Knight RM (2015) Transcriptomic analysis comparing stay-green and senescent Sorghum bicolor lines identifies a role for proline biosynthesis in the stay-green trait. J Exper Bot. doi:10.1093/jxb/erv405

Joo YK, Kim WY, Kwak KJ, Seung HO, Han YS, Kang H (2010) Glycine-rich RNA-binding proteins are functionally conserved in*Arabidopsis thaliana* and *Oryza sativa* during cold adaptation process. J Expl Bot. 61(9): 2317–2325.

Kim S, Lee J, Kim J, Young Sang Kwon YS, Dong-Won Bae D, Bae HH, Son B, Seong-Bum Baek S, Kwon Y, Woo M and Shin S (2015) Physiological and proteomic analysis of the response to drought stress in an inbred Korean maize line. Plant Omics J. 8(2):159-168.

Kiyosue T, Yamaguchi-Shinozaki K, Shinozaki K (1994) Cloning of cDNAs for genes that are early-responsive to dehydration stress (ERDs) in *Arabidopsis thaliana* L.: identification of three ERDs as HSP cognate genes. Plant Mole Biol. 25:791–798.

Komatsu S, Kamal AHM, Hossain Z (2014) Wheat proteomics: proteome modulation and abiotic stress acclimation. Fronr Plant Sci. 5:684. doi: 10.3389/fpls. 2014.00684

Kononowicz AK, Raghothama KG, Casa AM, Reuveni M, Watad AA, Liu D, Bressan RA, Hasegawa PM (1993) Osmotin: regulation of gene expression and function. *In*: Close TJ, Bray EA(ed) plant response to cellular dehydration during environmental stress. American Society of Plant Physiologists, Rockville, Maryland, USA.

Lindah M, Spetea C, Hundal T, Oppenheim AB, Adam Z, Andersson B (2000) The thylakoid FtsH protease plays a role in the light-induced turnover of the photosystem II D1 protein. Plant Cell. 12 (3):419-31.

Liu Y, Fiskum G, Schubert D (2002) Generation of reactive oxygen species by mitochondrial electron transport chain. Journal of Neurochemestry. 80: 780- 787.

Lux A, Luxová M, Hattori T, Inanaga S, Sugimoto Y. (2002).Silicification in sorghum (Sorghum bicolor) cultivars with different drought tolerance. Physiol Plant. 115(1):87-92.

Maheswari M, Varalaxmi Y, Vijayalakshmi A, Yadav SK, Sharmila P, Venkateswarlu B, Vanaja M, Pardha Saradhi P (2010) Metabolic engineering using mtlD gene enhances tolerance to water deficit and salinity in sorghum. Biology Plant. 54:647-652.

Mally A, Witt SN (2001) GrpE accelerates peptide binding and release from the high affinity state of DnaK. Nature Structural Biology. 8:254–257.

Manivannan P, Abdul Jaleel C, Sankar B, Kishorekumar A, Somasundaram, R, Lakshmanan GMA, Panneerselvam R (2007) Growth, biochemical modifications and proline metabolism in Helianthus annuus L. as induced by drought stress. Colloids and Surfaces B: Biointerfaces. 59: 141–149.

Ngara R, Ndimba R, Borch-Jensen J, Jensen ON, Ndimba B (2012) Identification and profiling of salinity stress-responsive proteins in Sorghum bicolor seedlings. J Proteom. *75*(13):4139–50.

Nyachiro JM, Briggs KG, Hoddinott J, Johnson-Flanagan AM (2001) Chlorophyll content, chlorophyll fluorescence and water deficit in spring wheat. Cereal Res Comm. 29: 135–142.

Ommen OE, Donnelly A, Vanhoutvin S, van Oijen M, Manderscheid R (1999) Chlorophyll content of spring wheat flag leaves grown under elevated CO2 concentrations and other environmental stresses within the ESPACE wheat project. Europ J Agron. 10: 197-203.

Peng ZY, Wang MC, Li F, Lu HJ, Li CL, et al. (2009) A Proteomic Study of the Response to Salinity and Drought Stress in an Introgression Strain of Bread Wheat. Mol Cell Proteom. 8: 2676– 2686.

Reviron MP, Vartanian N, Sallantin M, Huet JC, Pernollet JC, de Vienne D (1992) Characterization of a nove1 protein induced by progressive or rapid drought and salinity in Brassica napus leaves. Plant Physiol. 100: 1486-1493

Riccardi F, Gazeau P, Jacquemot MP, Vincent D, Zivy M (2004) Deciphering genetic variations of proteome responses to water deficit in maize leaves. Plant Physiol and Biochem. 42:1003–1011.

Salih AA, Ali IA, Lux A, Luxova M, Cohen Y, Sugimoto Y, Inanaga S (1999) Rooting, water uptake, and xylem structure adaptation to drought of two sorghum cultivars. Crop Sci. 39: 168-173.

Seki M, Narusaka M, Ishida J *et al.* (2002) Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J. 31:279–292.

Somerville C, Youngs H, Taylor C, Davis SC, Long SP (2010) Feedstocks for lignocellulosic biofuels. Sci. 329:790–792.

Stumpf PK (1984) in Fatty Acid Metabolism and Its Regulation (Numa, S. ed) p 155 179, Elsevier Science Publ., Amsterdam.

Tyagi A, Kumar N and Sairam RK (1999) Efficacy of RWC, Membrane Stability, Osmotic Potential, Endogenous ABA and Root Biomass as Indices for Selection against Water Stress in Rice. Ind J Plant Physiol. 4(4):302-306.

Van Wijk KJ (2001) Challenges and prospects of plant proteomics. Plant Physiol. 126: 501- 508.

Veeranagamallaiah G, Prasanthi J, Reddy KE, Pandurangaiah M, Babu OS, *et al.* (2011) Group 1 and 2 LEA protein expression correlates with a decrease in water stress induced protein aggregation in horsegram during germination and seedling growth. J Plant Physiol. 168: 671– 677.

Verbruggen N, Hermans C (2008) Proline accumulation in plants: a review. Amino Acids. 35: 753-759.

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci, 9:244–252.

Whitney SM, Andrews TJ (2001) The gene for the ribulose 1,5-bisphosphate Carboxylase/Oxygenase (Rubisco) small subunit relocated to the plastid genome of tobacco directs the synthesis of small subunits that assemble into rubisco. The Plant Cell. 13(1):193–206.

Williams J, Bulman M, Huttly A, Phillips A, Neill S (1994) Characterization of a cDNA from *Arabidopsis thaliana* encoding a potential thiol protease whose expression is induced independently by wilting and abscisic acid. Plant Mole Biol. 25:259–270.

Xu CP, Huang BR (2010) Comparative analysis of drought responsive proteins in Kentucky bluegrass cultivars contrasting in drought tolerance. Crop Sci. 50: 2543– 2552.

Yancy PH, Clark ME, Hand SC, Bowlus RD, Somero GN (1982) Living with water stress: evolution of osmolyte systems. Sci. 217: 1214–1223.

Yin H, Chen JC, Yang J, David J, Chen J, Muchero W, Ye N, Tschaplinski JT, Stan DW, Cheng Z, Tuskan AG, Yang X (2014) Functional genomics of drought tolerance in bioenergy crops. Crit Rev Plant Sci. 33:205–224.

Zhu JK, Hasegawa PM, Bressan RA (1997) Molecular aspects of osmotic stress in plants. Crit Rev Plant Sci.16:253–277.