Spanish Journal of Agricultural Research
17 (2), e0802, 12 pages (2019)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2019172-14300
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, O.A, M.P. (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Proteomic changes in the grains of foxtail millet (*Setaria italica* (L.) Beau) under drought stress

Jing Li[1]*, Xia Li[1,2]*, Qinghua Yang[1], Yan Luo[1], Xiangwei Gong[1], Weili Zhang[1], Yingang Hu[1], Tianyu Yang[3], Kongjun Dong[3] and Baili Feng[1]

[1]*Northwest A & F University, College of Agronomy, State Key Laboratory of Crop Stress Biology for Arid Areas, Yangling, Shaanxi Province, 712100 China.* [2]*Ankang Institute of Agricultural Sciences, Ankang, Shaanxi Province, 725021 China.* [3]*Gansu Academy of Agricultural Sciences, Crop Research Institute, Lanzhou, Gansu Province, 730070 China.*

**Jing Li and Xia Li contributed equally to this work.*

Abstract

Drought has become a serious problem that threatens global food security. Foxtail millet (*Setaria italica*) can be used as a model crop for drought-resistant research because of its excellent performance in drought tolerance. In this study, the typical drought-tolerant foxtail millet landrace 'Huangjinmiao' was grown in a field under control and drought stress conditions to investigate its response to drought stress. The proteins in the harvested grains were analysed through two-dimensional electrophoresis (2-DE) coupled with matrix-assisted laser desorption/ionization-tandem time-of-flight (MALDI-TOF/TOF) analysis to characterize the response of foxtail millet under drought stress at a proteomic level. A total of 104 differentially abundant protein spots (DAPs) were identified; among them, 57 were up-regulated and 47 were down-regulated under drought treatment. The identified proteins were involved in an extensive range of biological processes, including storage proteins, protein folding, starch and sucrose metabolism, glycolysis/gluconeogenesis, biosynthesis of amino acids, detoxification and defense, protein degradation, tricarboxylic acid (TCA) cycle, protein synthesis, energy metabolism, transporter, pentose phosphate pathway, and signal transduction. Post-translational protein modifications might also occur. Moreover, the albumin content greatly decreased under drought stress, whereas the gliadin content considerably increased (p<0.01). In conclusion, this study provides new information on the proteomic changes in foxtail millet under drought stress and a framework for further studies on the function of these identified proteins.

Additional keywords: DAPs; protein components; MALDI-TOF/TOF; 2-DE; post-translational modifications.

Abbreviations used: Ctrl (control); DAPs (differentially abundant protein spots); DS (drought stress); DTT (DL-dithiothreitol); IEF (isoelectric focusing); IPG (immobilized pH gradient); TCA (tricarboxylic acid).

Authors' contributions: Conceived and designed the experiments: JL, XL, and BLF. Performed the experiments: JL, XL, QHY, YL, XWG, and WLZ. Analyzed the data: JL, XL, YGH, and BLF. Contributed materials: TYY and KJD. Wrote the paper: JL and XL.

Citation: Li, J.; Li, X.; Yang, Q. H.; Luo, Y.; Gong, X. W.; Zhang, W. L.; Hu, Y. G.; Yang, T. Y.; Dong, K. J.; Feng, B. L. (2019). Proteomic changes in the grains of foxtail millet (*Setaria italica* (L.) Beau) under drought stress. Spanish Journal of Agricultural Research, Volume 17, Issue 2, e0802. https://doi.org/10.5424/sjar/2019172-14300

Supplementary material (Tables S1 and S2; Fig. S1) accompanies the paper on SJAR's website.

Received: 22 Nov 2018. **Accepted:** 17 May 2019.

Funding: National Natural Science Foundation of China (31371529); National Millet Crops R&D System (CARS-06-13.5-A26); Shaanxi Province Key Research and Development Projects (S2018-YF-TSLNY-0005); and Minor Grain Crops R&D System of Shaanxi Province (2018).

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Baili Feng: fengbaili@nwsuaf.edu.cn

Introduction

Drought has become a global problem that severely limits plant growth and crop productivity and threatens world food security (Li *et al.*, 2014; Chmielewska *et al.*, 2016; Wu *et al.*, 2016). This phenomenon has exacerbated global climate change (Abedi & Pakniyat, 2010; Taniguchi, 2016; Duran-Encalada *et al.*, 2017). According to the 4th Intergovernmental Panel on Climate Change (IPCC) report (Alley *et al.*, 2007), Earth is constantly warming, and the drought is gradually becoming a complicated issue; it has been reported that drought can result in 80% yield loss of the common bean (Zadraznik *et al.*, 2017). Food security is the foundation of human survival and social stability (Wang *et al.*, 2013), and China is a country with a huge population and heavily dependent on agriculture. Previous study has demonstrated that precipitation has been on the decline since the 1980s in North China (Ma *et al.*, 2005), and this finding has

prompted us to begin to actively respond to the impact of drought.

A clear understanding of the molecular mechanisms of plant response to drought is essential for plant biology. Knowledge about these mechanisms is also crucial for the continued development of rational breeding and transgenic strategies to improve stress tolerance in crops (Cui *et al.*, 2005). Therefore, overcoming the influence of drought on agricultural production has been the focus of research. Drought resistance is complex, and many proteins related to various metabolic processes are involved in the process (Ge *et al.*, 2012). So far, changes in physicochemical and molecular mechanisms come down to plants under drought stress have been reported. Proteins participating in chlorophyll biosynthesis (Wu *et al.*, 2016), nitrogen metabolism (Chmielewska *et al.*, 2016), defense and stress (Zadraznik *et al.*, 2017), storage starch biosynthesis (Ganeshan *et al.*, 2010), transcription and translation (Cao *et al.*, 2017) play a crucial role in drought response. In addition, previous studies have found that grain protein content, glutenin content and the ratio of glutelin to gliadin increased greatly under stress conditions (Lan *et al.*, 2004; Lu & Lu, 2013). Therefore, research on proteomic changes involved in drought stress plays an important role in understanding the mechanism of drought in plants.

Foxtail millet (*Setaria italica* (L.) Beau), an ancient crop that originated from China in 6000 BC, has gradually become an important food and fodder grain crop in arid and semi-arid regions in Asia, especially in China and India (Bettinger *et al.*, 2010; Wang *et al.*, 2016), because of its abundant proteins, dietary fibres, phenolics, flavonoids and minerals in its grain (Liu *et al.*, 2017) and its high tolerance to natural abiotic stress (Yadav *et al.*, 2016). Foxtail millet can be used as a model crop for drought resistance research because of its excellent performance in drought tolerance. It is characterized by a small genome, low amount of repetitive DNA, inbreeding nature and short life cycle (Lata *et al.*, 2013; Yi *et al.*, 2015). Therefore, foxtail millet plays a crucial role in plant research. Foxtail millet proteins are known to be involved in several NaCl stress-related progresses, such as signal transduction, photosynthesis, cell wall biogenesis, stress related and several metabolisms, including energy, lipid, nitrogen, carbohydrate and nucleotide metabolisms (Veeranagamallaiah *et al.*, 2008). Previous study also found that SiLEA14, a novel atypical late embryogenesis abundant (LEA) protein, plays important roles in resistance to abiotic stresses in foxtail millet (Wang *et al.*, 2014). To the best of our knowledge, the response of foxtail millet grains to drought stress at a proteomic level has yet to be reported. Therefore, investigating the response mechanisms of foxtail millet grain proteins under drought stress offers great theoretical and practical importance.

Material and methods

Plant materials and experimental design

'Huangjinmiao', a foxtail millet landrace widely cultivated in Northwestern China and characterized by high drought resistance and excellent quality, was used as the experimental material. The seeds used in the experiment were provided by the Chifeng Academy of Agriculture and Animal Husbandry, Chifeng, Inner Mongolia Autonomous Region, China. Experiments were conducted in Dunhuang, Gansu Province, China (92°13′-95°30′E, 39°40′-41°40′N, altitude 1139 m) in 2014. The area is characterized by a typical warm temperate arid climate with the following conditions: annual average precipitation of approximately 45.4 mm, annual evaporation of 2486 mm, dryness of 19.6%, average annual temperature of 10.2 °C, frost-free period of 142 days, and annual sunshine duration of 3246.7 h. In the experimental site, 0–40 cm soil showed a loess-like loam texture with 8.60 g kg^{-1} organic carbon, 20.83 mg kg^{-1} available nitrogen, 40.40 mg kg^{-1} available phosphorus and 112.79 mg kg^{-1} available potassium. The pH value of the soil was 7.90.

The study involved a completely randomized block design with three replications and two treatments, control (Ctrl) and drought stress (DS) treatment. Impervious belts of at least 1 m wide were set between treatments. Foxtail millet 'Huangjinmiao' was sown on April 18, 2014, and harvested on August 28, 2014. A sufficient amount of water was poured before the test to ensure that the seed germinated normally. For the control group, foxtail millet was irrigated once at each of the main growth stages of jointing stage, heading stage and filling stage. For drought stress treatment, the crops were not irrigated during the entire growing stages, and the source of water was completely dependent on soil moisture and precipitation. The temperature and precipitation amount during the foxtail millet growth period of 2014 were shown in Fig. 1. In the experiment, the fertilization was performed before sowing by using 354 kg ha^{-1} (received 165 kg N ha^{-1}) urea, 321.8 kg ha^{-1} (received 195 kg P_2O_5 ha^{-1}) calcium superphosphate and 194.4 kg ha^{-1} (received 105 kg K_2O ha^{-1}) potassium sulfate. During the growth period, no additional fertilizers were applied. During the maturation period of foxtail millet, the yield and yield-related indexes such as panicle weight per plant, grain weight per plant, stem and leaf weight per plant, the number of effective panicle and 1000-grain weight were investigated with

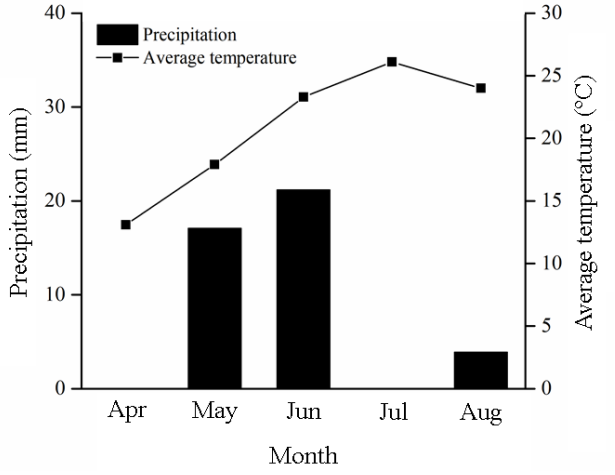

Figure 1. Monthly precipitation and average temperatures during the foxtail millet growth period in 2014. The bar chart represents the precipitation of each month, and the line chart illustrates the average temperature.

three replications. The harvested mature grains were used for further experiments.

Extraction and identification of protein components in foxtail millet grains

The protein components in the mature seeds of foxtail millet were extracted in accordance with the method of Agboola *et al.* (2005) with some modifications. Defatted grain powders (approx. 3 g) were added to a 50 mL centrifuge tube that was filled with water up to the 50 mL mark. The centrifuge tubes were placed on a table concentrator, shaken at room temperature for 3 h and centrifuged at 4500 rpm and at 4˚C for 40 min. Approximately 30 mL of the supernatant was considered as the test solution, then we added, following this order, 5% NaCI solution, 70% ethanol and NaOH solution, 1% sodium dodecyl sulfate (SDS) solution and 1%SDS+2% 2-mercapto-ethanol (2-ME) solution into the precipitate. Albumin, globulin, gliadin, glutelin, 1% SDS soluble protein and 1% SDS+2% 2-ME soluble protein were extracted by repeating the above procedures. The contents of protein components were determined by the Kjeldahl method (Kjeltec 8400 type Kjeldahl apparatus, FOSS Company, Denmark). The coefficient 6.25 was used for crude protein calculation (Yu *et al.*, 2007). Each experiment was performed in triplicate.

Extraction and quantification of total proteins

The total proteins in the mature seeds of foxtail millet were extracted in accordance with the methods of Wang *et al.* (2007) and Cui (2012). Approximately 1 g foxtail millet grains was weighted and then rapidly ground into powder after liquid nitrogen was added. The powder was transferred into a 10 mL centrifuge tube and we added five volumes of precooled trichloroacetic acid (TCA) extract containing 10% TCA-acetone, 0.07%-0.1% DL-dithiothreitol (DTT) and 0.5% beta-mercaptoethanol. The solution was mixed thoroughly by shaking, and then placed in a refrigerator at -20 ˚C overnight. The solution was centrifuged at 13,000 rpm and at 4 ˚C for 30 min and the supernatant was discarded. The precipitate was washed three times with pre-cooled acetone containing 0.07%-0.1% DTT and dried for 45 min under vacuum. Then we added to the dried powder 5 mL of protein extract solution (containing 0.5 mol·L^{-1} pH 7.5 tris-HCl, 30% sucrose, 50 mmol L^{-1} EDTA-Na_2, 2% SDS, 2% β-mercaptoethanol and 100 mmol L^{-1} KCl) and 5 mL of pH 7.8-8.0 tris-saturated phenol. The solution was bathed for 5 min after it was blended in warm water (50 ˚C), and centrifuged for 20 min; 1 mL of phenolic layer was pipetted into a 10 mL centrifuge tube, then we added 5 volumes of ammonium acetate solution containing 0.1% β-mercaptoethanol; and tubes were placed in a refrigerator -80 ˚C overnight. The solution was centrifuged at 12,000 g for 10 min at 4 ˚C and the supernatant was discarded. The precipitate was washed three times with pre-cooled methanol containing 0.1% β-mercaptoethanol, and stored in a refrigerator at -80˚C.

We added to the treated samples a hydrated solution consisting of 7 M urea, 2 M thiourea, 4% 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonate (CHAPS), 0.2% immobilized pH gradient (IPG) buffer and 65 mM DTT and allowed to crack in a water bath at 30 ˚C for 2 h. The samples were then centrifuged for 20 min, and the supernatant was pipetted into a cuvette to determine the protein concentration. Protein quantification was conducted via Bradford's method. A standard curve of bovine serum albumin was made, and absorbance was determined at 595 nm by using a DU800 spectrophotometer (Beckman Company, USA).

Two-dimensional electrophoresis (2-DE), image analysis and matrix-assisted laser desorption/ionization-tandem time-of-flight (MALDI-TOF/TOF) analysis

For the first dimension of electrophoresis, 0.001% bromophenol blue was added to 300 μL of 800 μg of hydrolyzed protein samples in a hydrated plate. The 17 cm IPG preformed strips were allowed to equilibrate to room temperature for 10 min and placed in a hydration plate surface down to avoid bubble formation. Subsequently, 5 mL of vegetable oil was added. Isoelectric focusing (IEF) was performed by applying

a voltage of 50 V for 14 h, 250 V for 30 min, 1000 V for 16 h and 8500 V for 5 h. A voltage of 10,000 V was maintained until a total of 80 kVh was reached. A voltage of 500 V was then maintained for 24 h. The second dimension of SDS-PAGE was performed immediately after IEF, and the strips were removed, balanced for 15 min in mother liquor twice, and placed above 11.5% gel. The strips were sealed with low-capacity and low-sodium agarose at 10 mA voltage for 40 min and set to 30 mA towards end.

After 2-DE was completed, the gel was stained with Coomassie brilliant blue (CBB) G-250 (Candiano *et al.*, 2004) and de-stained with double-distilled water. Each gel was then scanned using a UMAX PowerLook 2100XL image scanner (UMAX Systems GmbH, Willich, Germany) at a resolution of 300 dpi. The transmission mode was selected, and the picture was set to TIFF format. Image analysis was performed using PDQuest 8.0.1 (Bio-Rad Laboratories, Hercules, CA, USA), including automatic protein spot detection and matching, background elimination, protein spot manual editing and matching and protein spot abundance uniformity. Differential protein spots were subjected to qualitative and quantitative analysis. The number of differentially abundant protein spots (DAPs) should satisfy the t-test of 0.05 level and the multiple of change was more than 1.5 times including induced and missing protein spots simultaneously. The DAPs were manually excised from the gels and analyzed by Hoogen Biotech Co., Ltd., Shanghai, China.

Mass spectrometry data was analyzed by GPS 3.6 (Applied Biosystems) and Mascot 2.1 (Matrix Science). The detailed parameter settings were as follows: Database, NCBI; taxonomy, Viridiplantae (900091); type of search, peptide mass fingerprint (MS/MS Ion Search); enzyme, trypsin; fixed modifications, carbamidomethyl (C); mass values, monoisotopic; protein mass, unrestricted; peptide mass tolerance, ± 100 ppm; fragment mass tolerance, ± 0.4 Da; peptide charge state, 1+; maximum missed cleavages, 1; protein score confidence interval (CI%) ≥ 95.

Statistical analysis

Statistical analysis was performed in accordance with the method of Wu *et al.* (2015) with some modifications. The figures used in this article were drawn using Microsoft Office Excel 2016 coupled with OriginPro 2016 (OriginLab Corporation, Northampton, MA, USA). Comparisons were statistically evaluated ANOVA in SPSS 23.0 (SPSS Science, Chicago, IL, USA). Significance was set at $p<0.05$ or $p<0.01$.

Results

Changes in the indexes related to the yield of foxtail millet under drought stress

After the foxtail millet matured, the changes in the yield and yield-related indexes under Ctrl and DS treatments were investigated (Table 1). The results indicated that yield, panicle weight per plant, grain weight per plant, and 1000-grain weight greatly decreased ($p<0.01$) under DS and the number of effective panicle significantly decreased ($p<0.05$). The stem and leaf weight per plant also decreased but this decrease was not significant under DS.

Changes in protein components in foxtail millet grains under drought stress

The contents of four protein components (albumin, globulin, prolamin and glutelin) under Ctrl and DS conditions are shown in Fig. 2. The results indicate that the albumin content greatly decreased ($p<0.01$) under DS, whereas the gliadin content considerably increased ($p<0.01$). The contents of glutelin and globulin also increased but this increase was not significant under DS.

Identification of differentially abundant proteins under drought stress

The total protein of the matured seeds was extracted and subjected to 2-DE analysis with three independent biological replicates to investigate the proteomic responses of foxtail millet grains under DS (Fig. S1 [suppl]). More than 1000 protein spots were detected using PD-Quest 7.0, and a total of 104 protein spots whose abundance was significantly altered ($p<0.05$) by more than at least 1.5-fold (Fig. 3) were screened. Amongst these protein spots, 57 were up-regulated and 47 were down-regulated under the DS treatment. All of the DAPs were analyzed by MALDI-TOF/TOF

Table 1. Indexes related to the yield of foxtail millet under drought stress (DS) compared with those of the control (Ctrl).

Treatment	Ctrl	DS
Panicle weight/plant (g)	24.78	8.59**
Grain weight/plant (g)	20.22	4.54**
Stem and leaf weight/plant (g)	29.43	24.6
Number of effective panicles	1.27	0.57*
1000-grain weight (g)	2.88	2.63**
Yield (10^3 kg ha^{-1})	7.82	1.36**

*,**: statistical significance at 0.05 ($p<0.05$) and 0.01 ($p<0.01$), respectively, according to Duncan's test.

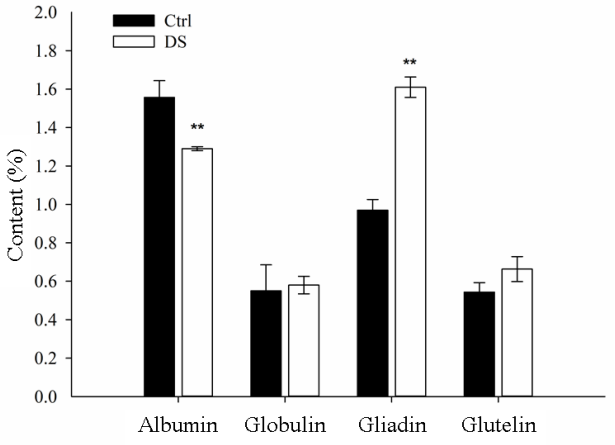

Figure 2. Content of protein components in foxtail millet grains under control (Ctrl) and drought stress (DS) treatment. Error bars represent standard deviation (n = 3). The ** above the histogram indicate statistical significance at 0.01 (p<0.01) according to Duncan's test.

and 104 DAPs were successfully identified (Table S1 [suppl]). A total of 73 proteins (duplicate proteins were not included) with known functions were finally obtained by searching the database. Amongst the 104 identified DAPs, 40 were classified into 12 kinds of proteins (Table 2), namely, 1,4-alpha-glucan-branching enzyme 2 (spots 13 and 71), glucose-1-phosphate adenylyltransferase small subunit (spots 17 and 31), heat shock 70 kDa protein (spots 21 and 84), fructose-bisphosphate aldolase cytoplasmic isozyme-like (spots 79, 83 and 85), protein disulfide-isomerase (spots 5, 11, 19 and 81), globulin-1 S allele-like (spots 6, 9, 48, 56, 59, 73, 74, 75, 82, 97, 99, 101 and 103), globulin-1 S allele, partial (spots 42, 44 and 93), glucose-1-phosphate adenylyltransferase large subunit 1 (spots 86 and 87), putative aconitate hydratase (spots 90 and 91), trypsin inhibitor (spots 10 and 95), glucose-1-phosphate adenylyltransferase (spots 62, 63 and 65), adenosine triphosphate (ATP) synthase F1 beta subunit (spots 32 and 35).

Functional classification and subcellular localization prediction of differentially abundant proteins under drought stress

Based on molecular function and metabolic pathway, these 104 DAPs were classified into 13 categories, including storage proteins (17.31% of the total 104 DAPs); protein folding (15.38% of the total 104 DAPs); starch and sucrose metabolism (14.42% of the total 104 DAPs); glycolysis/gluconeogenesis (10.58% of the total 104 DAPs); biosynthesis of amino acids, detoxification and defense, protein degradation and TCA cycle (6.73% each of the total 104 DAPs); protein synthesis (5.77% of the total 104 DAPs); energy metabolism (4.81% of the total 104 DAPs); transporter (2.88% of the total 104 DAPs); pentose phosphate pathway (0.96% of the total 104 DAPs) and signal transduction (0.96% of the total 104 DAPs). Approximately 50% of these identified proteins were involved in the first four functional groups. The regulation of differentially abundant proteins with different functional classification was diverse under DS (Fig. 4; Table S2 [suppl]). Most of the DAPs, that is, 50 and 20, were respectively located in

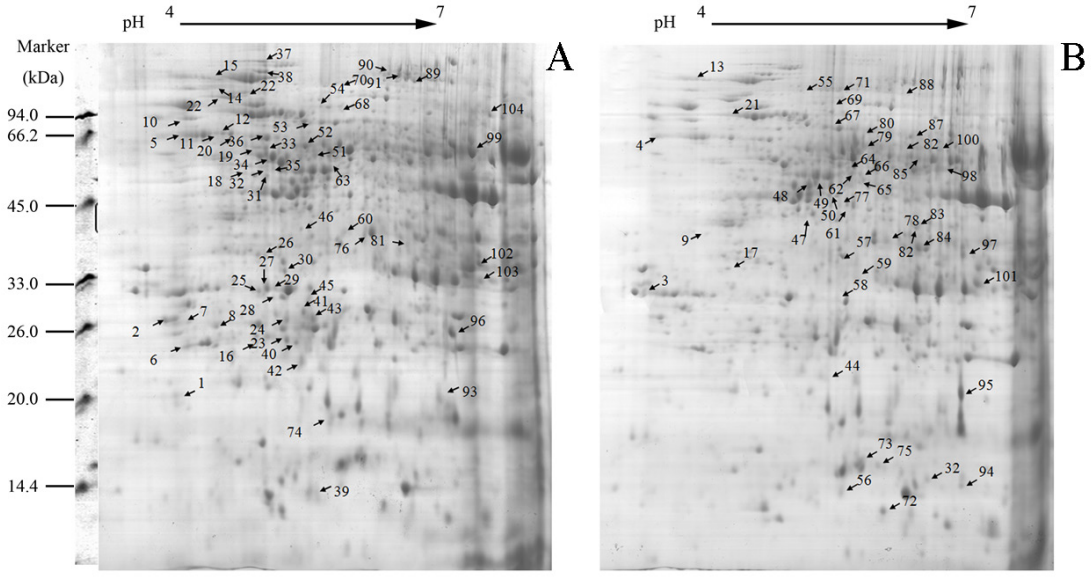

Figure 3. Representative two-dimensional gel electrophoresis graphs of foxtail millet grain proteins. Protein spots labelled with numbers on the map are identified through MALDI-TOF/TOF MS. A, Drought stress (DS); B, control (Ctrl).

Table 2. Partial differentially abundant protein spots identified as the same protein.

Protein	Spot No.	Accession No.	Theoretical/Experimental		Fold change	Subcellular location	Possible function
			Mw (kDa)	pI			
1,4-alpha-glucan-branching enzyme 2	13	gi|514712923	90.71/76.29	5.72/5.24	20.70	amyloplastic	Starch and sucrose metabolism
	71	gi|514712923	90.71/89.03	5.72/5.76	4.88	amyloplastic	Starch and sucrose metabolism
glucose-1-phosphate adenylyltransferase small subunit	17	gi|514728628	56.10/52.79	5.92/5.18	2.80	amyloplastic	Starch and sucrose metabolism
	31	gi|514728628	56.10/56.67	5.92/5.73	-2.11	amyloplastic	Starch and sucrose metabolism
heat shock 70 kDa protein	21	gi|514784173	71.23/70.89	5.10/4.98	8.61	cytoplasmic	Protein Folding
	84	gi|514784173	71.23/70.39	5.10/4.96	2.53	cytoplasmic	Storage proteins
fructose-bisphosphate aldolase cytoplasmic isozyme-like	79	gi|514785954	39.19/42.31	6.56/6.38	46.59	cytoplasmic	Glycolysis / Gluconeogenesis
	83	gi|514785954	39.19/40.86	6.56/6.43	29.09	cytoplasmic	Glycolysis / Gluconeogenesis
	85	gi|514785954	39.19/37.69	6.56/6.49	2.45	cytoplasmic	Glycolysis / Gluconeogenesis
protein disulfide-isomerase	5	gi|514808170	56.75/56.39	4.90/4.77	-3.95	endoplasmic reticulum	Protein Folding
	11	gi|514808170	56.75/57.24	4.90/4.95	-5.03	endoplasmic reticulum	Protein Folding
	19	gi|514808170	56.75/55.69	4.90/5.76	-12.86	endoplasmic reticulum	Protein Folding
	81	gi|514808170	56.75/57.26	4.90/4.28	-9.06	endoplasmic reticulum	Protein Folding
globulin-1 S allele-like	6	gi|514813383	50.63/58.26	6.39/6.07	-2.65	vacuole	Storage proteins
	9	gi|514813383	50.63/38.79	6.39/5.49	4.41	vacuole	Storage proteins
	48	gi|514813383	50.63/41.38	6.39/7.38	2.99	vacuole	Storage proteins
	56	gi|514813383	50.63/43.17	6.39/6.23	2.44	vacuole	Storage proteins
	59	gi|514813383	50.63/52.04	6.39/6.47	76.84	vacuole	Storage proteins
	73	gi|514813383	50.63/23.88	6.39/6.28	4.49	vacuole	Storage proteins
	74	gi|514813383	50.63/46.21	6.39/6.09	-178.63	vacuole	Storage proteins
	75	gi|514813383	50.63/60.27	6.39/6.08	18.66	vacuole	Storage proteins
	82	gi|514813383	50.63/51.04	6.39/6.34	2.66	vacuole	Storage proteins
	97	gi|514813383	50.63/52.04	6.39/6.42	5.28	vacuole	Storage proteins
	99	gi|514813383	50.63/39.26	6.39/6.28	-4.00	vacuole	Storage proteins
	101	gi|514813383	50.63/47.06	6.39/6.21	2.44	vacuole	Storage proteins
	103	gi|514813383	50.63/38.29	6.39/5.10	-2.29	vacuole	Storage proteins
globulin-1 S allele, partial	42	gi|514819529	55.60/45.16	7.66/6.23	-2.99	vacuole	Storage proteins
	44	gi|514819529	55.60/54.98	7.66/7.65	3.96	vacuole	Storage proteins
	93	gi|514819529	55.60/66.31	7.66/7.58	-4.01	vacuole	Storage proteins
glucose-1-phosphate adenylyltransferase large subunit 1	86	gi|835968826	58.10/67.36	6.08/6.13	6.16	amyloplastic	Starch and sucrose metabolism
	87	gi|835968826	58.10/69.06	6.08/6.05	5.40	amyloplastic	Starch and sucrose metabolism
putative aconitate hydratase	90	gi|835993763	107.13/105.36	6.89/6.82	-2.17	cytoplasmic	TCA cycle
	91	gi|835993763	107.13/106.26	6.89/6.76	-2.20	cytoplasmic	TCA cycle
trypsin inhibitor	10	gi|944233813	22.95/24.06	6.35/6.26	-4.22	cytoplasmic	Protein degradation
	95	gi|944233813	22.95/23.37	6.35/6.19	195.53	cytoplasmic	Protein degradation

Table 2. Continued.

Protein	Spot No.	Accession No.	Theoretical/Experimental		Fold change	Subcellular location	Possible function
			Mw (kDa)	pI			
glucose-1-phosphate adenylyltransferase	62	gi|944237145	52.79/53.39	5.51/4.87	2.44	cytoplasmic	Starch and sucrose metabolism
	63	gi|944237145	52.79/52.19	5.51/5.37	33.28	cytoplasmic	Starch and sucrose metabolism
	65	gi|944237145	52.79/53.39	5.51/5.28	3.56	cytoplasmic	Starch and sucrose metabolism
ATP synthase F1 beta subunit	32	gi|944242149	66.71/67.09	5.70/5.71	-3.81	cytoplasmic	Energy metabolism
	35	gi|944242149	66.70/65.59	5.70/5.63	-3.02	cytoplasmic	Energy metabolism

cytoplasmic and vacuole based on subcellular localization prediction (Fig. 5).

A total of 18 proteins related to storage proteins were identified. Amongst them, 7 were up-regulated and 11 were down-regulated. Plant carbon metabolism generally included glycolysis, TCA cycle and carbohydrate metabolism. A total of 11 DAPs were involved in the glycolysis pathway. Of these proteins, 5 were up-regulated and 6 were down-regulated. At the same time, there were 7 proteins associated with the TCA cycle, 6 were up-regulated and 15 were involved in starch synthesis and metabolism, with 9 of them were down-regulated under DS. Further analysis showed that there were 6 proteins involved in protein synthesis, 16 proteins were involved in protein folding, and 7 proteins were associated with protein degradation, which were collectively referred to as grain proteins associated with protein metabolism. Amongst the 16 proteins involved in protein folding under drought treatment, 9 were up-regulated and 7 were down-regulated. Four proteins were up-regulated among the 7 proteins involved in protein degradation. Furthermore, 7 proteins were involved in amino acid-related metabolism, with 5 of them were down-regulated and 2 were up-regulated. There were 7 proteins related to detoxification and defense, with 5 of them were up-regulated. Five proteins related to energy metabolism were identified and all of them were up-regulated under DS. In addition, there were 3 proteins, 1 protein, and 1 protein related to transporter, pentose phosphate pathway, signal transduction, respectively, and they were all up-regulated under DS (Table S1 [suppl]).

Discussion

Changes in yield and protein components under drought stress

Drought stress has a great impact on crop yield and quality (Candogan *et al.*, 2013). Similar to previous findings (Samarah *et al.*, 2009; Kilic & Yağbasanlar, 2010; Li *et al.*, 2011), this study indicates that yield and yield-related indexes such as panicle weight per plant, grain weight per plant, 1000-grain weight, and the number of effective panicle, are greatly affected by DS. The protein is one of the important qualities of foxtail millet. Cereal properties and quality are significantly affected by grain protein content and its composition (Pierre *et al.*, 2007; Sunilkumar & Tareke, 2016), and DS drastically affects crops at the transcriptional level of genes encoding storage proteins (Begcy & Walia, 2015). In this study, the protein components of foxtail millet grains were measured under different irrigation treatments and found that different protein components behaved differently. Under the Ctrl condition, the following protein components of foxtail millet grains at high to low contents were obtained: albumin (1.56%), gliadin (0.97%), globulin (0.55%) and glutelin (0.54%), the result was consistent with a previous study (Liu *et al.*, 2014). Nevertheless, the highest content under DS was observed in gliadin (1.61%), followed by albumin (1.29%), glutelin (0.66%) and globulin (0.58%). The content of gliadin under DS was significantly higher than that under Ctrl condition, which was similar to the content in wheat (Shen *et al.*, 2006) and rice (Lu, 2012). The ratio of glutelin to gliadin was 0.56 under DS treatment whereas the ratio of glutelin to gliadin was 0.41 under the Ctrl treatment, which was inconsistent with that in wheat (Lan *et al.*, 2004) but was consistent with that in winter wheat (Dai *et al.*, 2006). Previous studies had shown that changes in flour quality of wheat were causally related to changes in protein composition under DS, and grain yield was significantly negatively correlated with protein content mainly because of high rates of grain N accumulation and low rates of carbohydrate accumulation under DS (Saint Pierre *et al.*, 2008; Candogan *et al.*, 2013). In this study, the protein components except albumin were negatively correlated with grain yield under DS. Investigating the proteomic changes in the grains

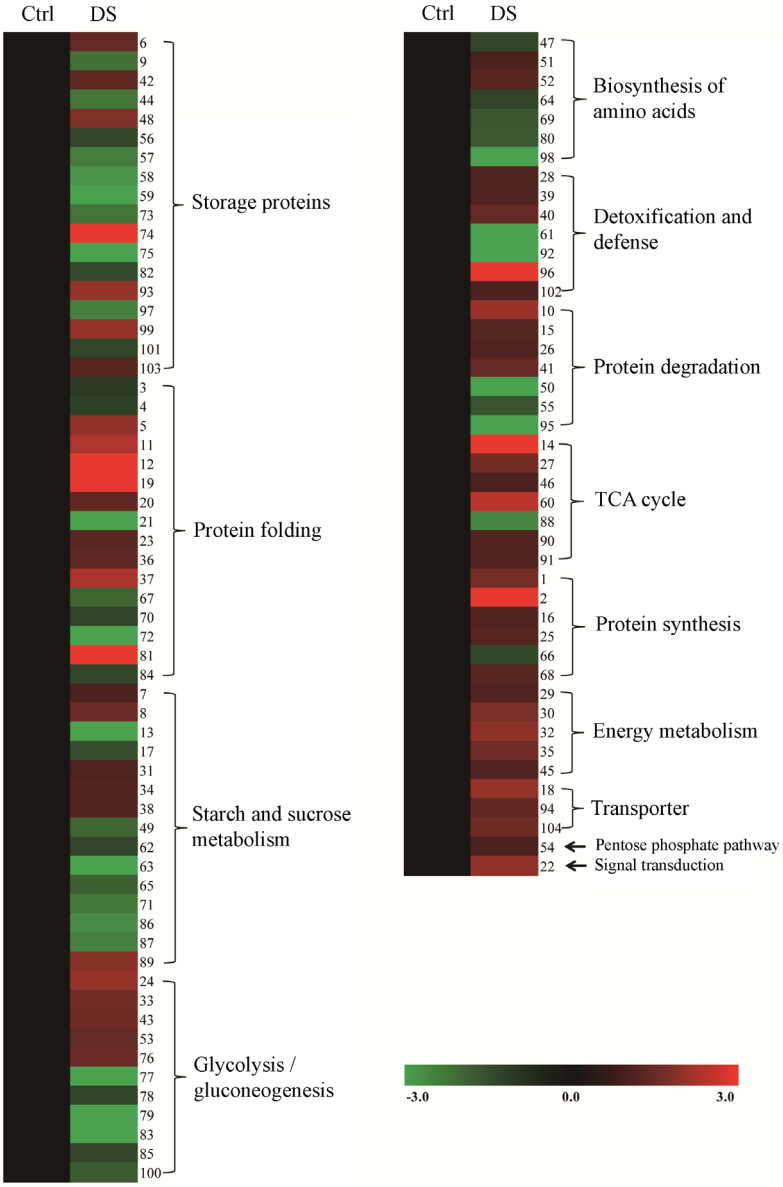

Figure 4. Clustering analysis of differentially abundant proteins under drought stress (DS) and control (Ctrl). The relative abundance value of each differentially abundant protein is represented by a bar with a specific colour. The up-regulated and down-regulated proteins are expressed as red and green, respectively. As the abundance value increases, the colour of the bar covers green to red, and when the abundance value is zero, the colour of the bar is black as shown in the bar at the bottom right.

would help comprehensively elucidate the effects of DS on foxtail millet.

Post-translational modification of proteins under drought stress

Proteomic approaches have emerged as efficient tools to study plant responses to stress (Veeranagamallaiah *et al.*, 2008). These approaches have identified not only changes in protein abundance, but also proteins that change through post-translational modifications (Mann & Jensen, 2003). In this study, some proteins were shown in different positions in 2-DE but were identified as the same protein, indicating the possibility of post-translational protein modification or degradation, such as proline hydroxylation, glycosylation, phosphorylation and proteolytic cleavage in foxtail millet grains under DS. This result was similar to previous findings (Holmes-Davis *et al.*, 2005; Gao & Wu, 2009; Simon, 2010). Globulin, a storage protein, was the only protein

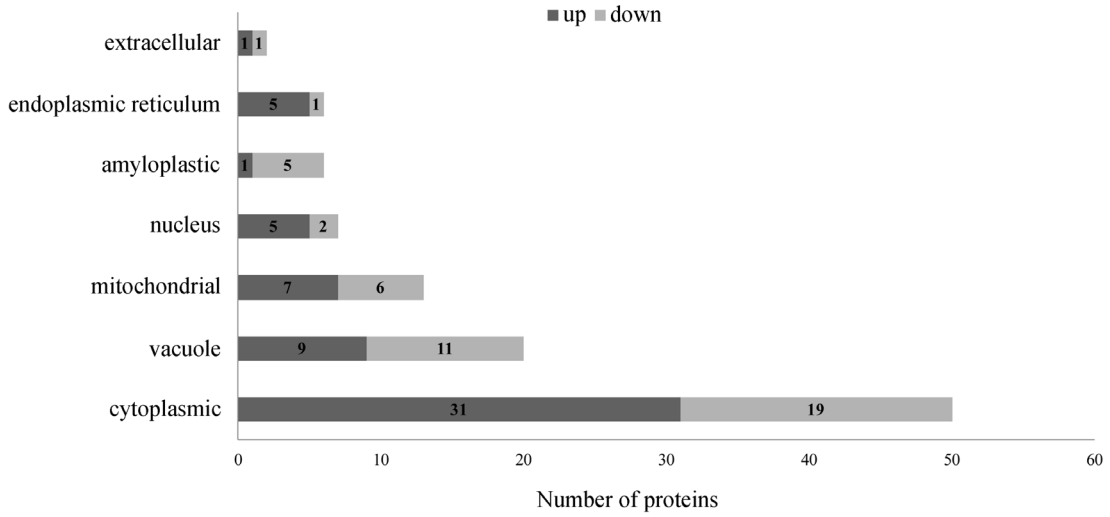

Figure 5. Subcellular localization of the differentially abundant proteins in foxtail millet grains under drought stress (DS). Black and grey bars represent the up-regulated and down-regulated proteins, respectively.

component that detected as DAPs through 2-DE. Of the 18 storage proteins, 16 were globulins. Of these globulins, 7 were up-regulated and 9 were down-regulated under DS. Therefore, the overall change in globulin was not evident, and this observation was consistent with the previous determination of protein components. Post-translational modifications could regulate protein activity, localization and protein–protein interactions in numerous cellular processes, thus leading to the elaborate regulation of plant responses to various external stimuli (Hashiguchi & Komatsu, 2016). Proteins which were present in multiple spots could be attributed to translation from alternatively spliced mRNAs (Ishikawa *et al.*, 1997). Veeranagamallaiah *et al.* (2008) showed that post-translational modifications can change the molecular weights and/or charge of proteins. Studies on post-translational modifications under stress conditions have been conducted in various crops, such as rice (Chastain & Gu, 2006), wheat (Zhang *et al.*, 2014), maize (Zörb *et al.*, 2010), sugar beet (Bing *et al.*, 2016) and canola (Koh *et al.*, 2015). To the best of our knowledge, foxtail millet under DS has not been investigated. Therefore, the post-translational modification mechanisms of foxtail millet should be intensively studied.

Proteins related to protein metabolism under drought stress

Protein metabolism generally included protein folding, protein synthesis and protein degradation. In this study, of the 29 DAPs involved in protein metabolism, 18 were up-regulated and 11 were down-regulated, indicating that protein metabolism was considerably enhanced to some extent under DS. Of the DAPs related to protein folding, 4 were heat shock related proteins (Spots 21, 37, 67 and 84). In general, heat-shock proteins were known for their overexpression in response to chemical and physiological stresses (Wu, 1995). Heat-shock proteins played an important role in normal cellular homeostasis and stress response (Kregel, 2002). They participated in protein folding, assembly, translocation and degradation in many normal cellular processes, stabilize proteins and membranes, and could assist in protein refolding under stressful conditions (Wang *et al.*, 2004). Genomic investigations have shown that heat-shock proteins are highly expressed in DS-exposed tissues (Cho & Hong, 2004). Furthermore, 5 of the 7 protein spots involved in protein degradation were proteasomes (Spots 15 and 26) that were up-regulated under DS. The degradation of many cytosolic and nuclear proteins and signalling pathways are affected by the proteasome pathway (Callis & Vierstra, 2000; Criqui *et al.*, 2002) and implicated in plant defense reactions (Suty *et al.*, 2003). Of the 6 DAPs related to protein synthesis, 5 were up-regulated under DS, indicating that drought greatly affected protein synthesis. These results demonstrate that DS greatly influenced protein metabolism.

Proteins related to energy metabolism under drought stress

In clustering heat map (Fig. 4), all of the DAPs related to energy metabolism were up-regulated. The result was consistent with previous studies showing that the abundance of ATP synthesis related proteins was greatly enhanced under stressful conditions (Parker *et al.*, 2006; Guo *et al.*, 2012). According to the study of Kottapalli *et al.* (2009), water deficit stress in barley

may be alleviated by inducing ATP synthases. ATP synthase F1 beta subunit coordinated ATP production with the demand for ATP-fuelled calcium pump activity and regulates cytosolic calcium concentrations (Hubbard & Mchugh, 1996).

In conclusion, this study aimed to characterize the response of foxtail millet under DS at a proteomic level. The results showed that DS greatly influenced foxtail millet in terms of its various properties, such as yield, yield-related indexes and protein components. A total of 104 DAPs were successfully identified through 2-DE coupled with MALDI-TOF/TOF analysis. Amongst them, 57 were up-regulated and 47 were down-regulated under DS. Notably, the identified proteins were involved in various biological processes, such as storage proteins, protein folding and starch and sucrose metabolism. Post-translational protein modifications might also occur. This study revealed changes in the protein components and proteome levels of foxtail millet grains under DS. However, a comprehensive analysis should be performed to further understand the variability in the response of foxtail millet to DS.

References

Abedi T, Pakniyat H, 2010. Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). Czech J Genet Plant Breed 46 (1): 27-34. https://doi.org/10.17221/67/2009-CJGPB

Agboola S, Ng D, Mills D, 2005. Characterisation and functional properties of Australian rice protein isolates. J Cereal Sci 41 (3): 283-290. https://doi.org/10.1016/j.jcs.2004.10.007

Alley RB, Berntsen T, Bindoff NL, Chen Z, Chidthaisong A, Friedlingstein P, Gregory JM, Hegerl GC, Heimann M, Hewitson B, 2007. IPCC, 2007: Summary for policymakers. Cambridge University Press.

Begcy K, Walia H, 2015. Drought stress delays endosperm development and misregulates genes associated with cytoskeleton organization and grain quality proteins in developing wheat seeds. Plant Sci 240: 109-119. https://doi.org/10.1016/j.plantsci.2015.08.024

Bettinger RL, Barton L, Morgan C, 2010. The origins of food production in north China: A different kind of agricultural revolution. Evol Anthropol 19 (1): 9-21. https://doi.org/10.1002/evan.20236

Bing Y, Li J, Jin K, Dufresne C, Na Y, Qi S, Zhang Y, Ma C, Duong BV, Chen S, 2016. Quantitative proteomics and phosphoproteomics of sugar beet monosomic addition line M14 in response to salt stress. J Proteom 143: 286-297. https://doi.org/10.1016/j.jprot.2016.04.011

Callis J, Vierstra RD, 2000. Protein degradation in signaling. Curr Opin Plant Biol 3 (5): 381-386. https://doi.org/10.1016/S1369-5266(00)00100-X

Candiano G, Bruschi M, Musante L, Santucci L, Ghiggeri GM, Carnemolla B, Orecchia P, Zardi L, Righetti PG, 2004. Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis 25 (9): 1327-1333. https://doi.org/10.1002/elps.200305844

Candogan BN, Sincik M, Buyukcangaz H, Demirtas C, Goksoy AT, Yazgan S, 2013. Yield, quality and crop water stress index relationships for deficit-irrigated soybean [*Glycine max* (L.) Merr.] in sub-humid climatic conditions Agr Water Manage 118: 113-121. https://doi.org/10.1016/j.agwat.2012.11.021

Cao Y, Luo Q, Tian Y, Meng F, 2017. Physiological and proteomic analyses of the drought stress response in *Amygdalus Mira* (Koehne) Yu et Lu roots. BMC Plant Biol 17 (1): 53. https://doi.org/10.1186/s12870-017-1000-z

Chastain CJ, Gu XY, 2006. Posttranslational regulation of pyruvate, orthophosphate dikinase in developing rice (*Oryza sativa*) seeds. Planta 224 (4): 924-934. https://doi.org/10.1007/s00425-006-0259-3

Chmielewska K, Rodziewicz P, Swarcewicz B, Sawikowska A, Krajewski P, Marczak L, Ciesiolka D, Kuczynska A, Mikolajczak K, Ogrodowicz P, *et al.*, 2016. Analysis of drought-induced proteomic and metabolomic changes in barley (*Hordeum vulgare* L.) leaves and roots unravels some aspects of biochemical mechanisms involved in drought tolerance. Front Plant Sci 7: 1108. https://doi.org/10.3389/fpls.2016.01108

Cho EK, Hong CB, 2004. Molecular cloning and expression pattern analyses of heat shock protein 70 genes from *Nicotiana tabacum*. J Plant Biol 47 (2): 149-159. https://doi.org/10.1007/BF03030646

Criqui MC, De AEJ, Camasses A, Capron A, Parmentier Y, Inzé D, Genschik P, 2002. Molecular characterization of plant ubiquitin-conjugating enzymes belonging to the UbcP4/E2-C/UBCx/UbcH10 gene family. Plant Physiol 130 (3): 1230. https://doi.org/10.1104/pp.011353

Cui S, Huang F, Wang J, Ma X, Cheng Y, Liu J, 2005. A proteomic analysis of cold stress responses in rice seedlings. Proteomics 5 (12): 3162-3172. https://doi.org/10.1002/pmic.200401148

Cui Y, 2012. Analysis of differential proteins of cotton leaves under salt stress. Mol Plant Breed 13 (4): 455.

Dai TB, Zhao H, Jing Q, Jiang D, Cao WX, 2006. Effects of high temperature and water stress during grain filling on grain protein and starch formation in winter wheat. Acta Ecologica Sinica 26 (11): 3670-3676.

Duran-Encalada JA, Paucar-Caceres A, Bandala ER, Wright GH, 2017. The impact of global climate change on water quantity and quality: A system dynamics approach to the US-Mexican transborder region. Eur J Oper Res 256 (2): 567-581. https://doi.org/10.1016/j.ejor.2016.06.016

Ganeshan S, Drinkwater JM, Repellin A, Chibbar RN, 2010. Selected carbohydrate metabolism genes show coincident expression peaks in grains of in vitro-cultured immature

spikes of wheat (*Triticum aestivum* L.). J Agric Food Chem 58 (7): 4193-4201. https://doi.org/10.1021/jf903861q

Gao Y, Wu QY, 2009. Identification of the proteomic changes in Synechocystis sp. PCC 6803 following prolonged UV-B irradiation. J Exp Bot 60 (4): 1141. https://doi.org/10.1093/jxb/ern356

Ge P, Ma C, Wang S, Gao L, Li X, Guo G, Ma W, Yan Y, 2012. Comparative proteomic analysis of grain development in two spring wheat varieties under drought stress. Anal Bioanal Chem 402 (3): 1297-1313. https://doi.org/10.1007/s00216-011-5532-z

Guo G, Ge P, Ma C, Li X, Lv D, Wang S, Ma W, Yan Y, 2012. Comparative proteomic analysis of salt response proteins in seedling roots of two wheat varieties. J Proteomics 75 (6): 1867. https://doi.org/10.1016/j.jprot.2011.12.032

Hashiguchi A, Komatsu S, 2016. Impact of post-translational modifications of crop proteins under abiotic stress. Proteomes 4 (4): 42. https://doi.org/10.3390/proteomes4040042

Holmes-Davis R, Tanaka CK, Vensel WH, Hurkman WJ, Mccormick S, 2005. Proteome mapping of mature pollen of *Arabidopsis thaliana*. Proteomics 5 (18): 4864-4884. https://doi.org/10.1002/pmic.200402011

Hubbard MJ, Mchugh NJ, 1996. Mitochondrial ATP synthase F1-beta-subunit is a calcium-binding protein. Febs Letters 391 (3): 323. https://doi.org/10.1016/0014-5793(96)00767-3

Ishikawa T, Yoshimura K, Tamoi M, Takeda T, Shigeoka S, 1997. Alternative mRNA splicing of 3'-terminal exons generates ascorbate peroxidase isoenzymes in spinach (*Spinacia oleracea*) chloroplasts. Biochem J 328 (3): 795-800. https://doi.org/10.1042/bj3280795

Kilic H, Yağbasanlar T, 2010. The effect of drought stress on grain yield, yield components and some quality traits of durum wheat (*Triticum turgidum* ssp. *durum*) cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38: 164-170.

Koh J, Chen G, Yoo MJ, Zhu N, Dufresne D, Erickson JE, Shao H, Chen S, 2015. Comparative proteomic analysis of *Brassica napus* in response to drought stress. J Proteome Res 14 (8): 3068-3081. https://doi.org/10.1021/pr501323d

Kottapalli KR, Rakwal R, Shibato J, Burow G, Tissue D, Burke J, Puppala N, Burow M, Payton P, 2009. Physiology and proteomics of the water-deficit stress response in three contrasting peanut genotypes. Plant Cell Environ 32 (4): 380-407. https://doi.org/10.1111/j.1365-3040.2009.01933.x

Kregel K, 2002. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol 92(5): 2177-2186. https://doi.org/10.1152/japplphysiol.01267.2001

Lan T, Jiang D, Xie ZJ, Dai TB, Jing Q, Cao WX, 2004. Effects of post-anthesis drought and waterlogging on grain quality traits in different specialty wheat varieties. J Soil Water Conserv 18 (1): 193-196.

Lata C, Gupta S, Prasad M, 2013. Foxtail millet: a model crop for genetic and genomic studies in bioenergy grasses. Crit Rev Biotechnol 33 (3): 328-343. https://doi.org/10.3109/07388551.2012.716809

Li C, Yue J, Wu X, Xu C, Yu J, 2014. An ABA-responsive DRE-binding protein gene from *Setaria italica*, SiARDP, the target gene of SiAREB, plays a critical role under drought stress. J Exp Bot 65 (18): 5415-5427. https://doi.org/10.1093/jxb/eru302

Li P, Chen J, Wu P, 2011. Agronomic characteristics and grain yield of 30 spring wheat genotypes under drought stress and nonstress conditions. Agron J 103: 1619-1628. https://doi.org/10.2134/agronj2011.0013

Liu J, Zhang Y, Liu Y, Zhang A, Zhao W, Li S, 2014. Analysis of protein components in foxtail millet. Food & Machinery 6: 39-42. (in Chinese).

Liu K, Qi S, Li D, Jin C, Gao C, Duan S, Feng B, Chen M, 2017. TRANSPARENT TESTA GLABRA 1 ubiquitously regulates plant growth and development from *Arabidopsis* to foxtail millet (*Setaria italica*). Plant Sci 254: 60-69. https://doi.org/10.1016/j.plantsci.2016.10.010

Lu D, Lu W, 2013. Effects of heat stress during grain filling on the functional properties of flour from fresh waxy maize. Cereal Chem 90: 65-69. https://doi.org/10.1094/CCHEM-03-12-0035-R

Lu X, 2012. Effects of water stress on protein component of rice grain. Food & Machinery 28 (5): 63-65. (in Chinese).

Ma Z, Huang G, Gan W, 2005. Multi-scale temporal characteristics of the dryness/wetness over northern China during the last century. Chinese Journal of Atmospheric Sciences 29 (5): 671-682. (in Chinese).

Mann M, Jensen ON, 2003. Proteomic analysis of post-translational modifications. Nature Biotechnol 21 (3): 255. https://doi.org/10.1038/nbt0303-255

Parker R, Flowers T, Moore A, Harpham N, 2006. An accurate and reproducible method for proteome profiling of the effects of salt stress in the rice leaf lamina. J Exp Bot 57 (5): 1109. https://doi.org/10.1093/jxb/erj134

Pierre CS, Peterson CJ, Ross AS, Ohm J, Verhoeven MC, Larson M, Hoefer B, 2007. Change in grain protein composition of winter wheat cultivars under different levels of N and water stress. In: Wheat production in stressed environments. Springer, Dordrecht, pp: 535-542. https://doi.org/10.1007/1-4020-5497-1_65

Saint Pierre C, Peterson CJ, Ross AS, Ohm JB, Verhoeven MC, Larson M, Hoefer B, 2008. Winter wheat genotypes under different levels of nitrogen and water stress: Changes in grain protein composition. J Cereal Sci 47: 407-416. https://doi.org/10.1016/j.jcs.2007.05.007

Samarah NH, Alqudah AM, Amayreh JA, McAndrews GM, 2009. The effect of late-terminal drought stress on yield components of four barley cultivars. J Agron Crop Sci 195: 427-441. https://doi.org/10.1111/j.1439-037X.2009.00387.x

Shen YX, Guo WS, Zhou Y, Zhu XK, Feng CN, Peng YX, 2006. Effects of salinity stress on the dynamic changes in the accumulation of grain protein and its components in wheat. Journal of Triticeae Crops 26 (6): 100-103. (in Chinese).

Simon WJ, 2010. Identification of *Arabidopsis* salt and osmotic stress responsive proteins using two-dimensional difference gel electrophoresis and mass spectrometry. Proteomics 5 (16): 4185-4196. https://doi.org/10.1002/pmic.200401282

Sunilkumar BA, Tareke E, 2016. Identification of discrepancies in grain quality and grain protein composition through avenin proteins of oat after an effort to increase protein content. Agr Food Security 5: 7. https://doi.org/10.1186/s40066-016-0056-6

Suty L, Lequeu J, Lançon A, Etienne P, Petitot AS, Blein JP, 2003. Preferential induction of 20S proteasome subunits during elicitation of plant defense reactions: towards the characterization of "plant defense proteasomes". Int J Biochem Cell Biol 35 (5): 637-650. https://doi.org/10.1016/S1357-2725(02)00386-2

Taniguchi K, 2016. Future changes in precipitation and water resources for Kanto Region in Japan after application of pseudo global warming method and dynamical downscaling. J Hydrol: Reg Stud 8: 287-303. https://doi.org/10.1016/j.ejrh.2016.10.004

Veeranagamallaiah G, Jyothsnakumari G, Thippeswamy M, Chandra Obul Reddy P, Surabhi GK, Sriranganayakulu G, Mahesh Y, Rajasekhar B, Madhurarekha C, Sudhakar C, 2008. Proteomic analysis of salt stress responses in foxtail millet (*Setaria italica* L. cv. Prasad) seedlings. Plant Sci 175 (5): 631-641. https://doi.org/10.1016/j.plantsci.2008.06.017

Wang M, Li P, Li C, Pan Y, Jiang X, Zhu D, Zhao Q, Yu J, 2014. SiLEA14, a novel atypical LEA protein, confers abiotic stress resistance in foxtail millet. BMC Plant Biol 14 (1): 1-16. https://doi.org/10.1186/s12870-014-0290-7

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 (5): 244-252. https://doi.org/10.1016/j.tplants.2004.03.006

Wang X, Jun MA, Zhang GY, Xi-Huan LI, Wang XF, Zhi-Ying MA, 2007. Proteomic analysis of cotton leaf under Verticillium dahliae stress. Cotton Sci 19 (4): 273-278. (in Chinese).

Wang Y, Li L, Tang S, Liu J, Zhang H, Zhi H, Jia G, Diao X, 2016. Combined small RNA and degradome sequencing to identify miRNAs and their targets in response to drought in foxtail millet. BMC Genet 17: 57. https://doi.org/10.1186/s12863-016-0364-7

Wang Z, He F, Fang W, Liao Y, 2013. Assessment of physical vulnerability to agricultural drought in China. Natural Hazards 67 (2): 645-657. https://doi.org/10.1007/s11069-013-0594-1

Wu C, 1995. Heat shock transcription factors: structure and regulation. Ann Rev Cell Devel Biol 11 (11): 441. https://doi.org/10.1146/annurev.cb.11.110195.002301

Wu K, Liu H, Sultan MARF, Liu Xl, Zhang J, Yu F, Zhao Hx, 2015. Physiological and comparative proteomic analysis reveals different drought responses in roots and leaves of drought-tolerant wild wheat (*Triticum boeoticum*). PLoS One 10: e0121852. https://doi.org/10.1371/journal.pone.0121852

Wu Y, Mirzaei M, Pascovici D, Chick JM, Atwell BJ, Haynes PA, 2016. Quantitative proteomic analysis of two different rice varieties reveals that drought tolerance is correlated with reduced abundance of photosynthetic machinery and increased abundance of ClpD1 protease. J Proteomics 143: 73-82. https://doi.org/10.1016/j.jprot.2016.05.014

Yadav A, Khan Y, Prasad M, 2016. Dehydration-responsive miRNAs in foxtail millet: genome-wide identification, characterization and expression profiling. Planta 243 (3): 749-766. https://doi.org/10.1007/s00425-015-2437-7

Yi F, Chen J, Yu J, 2015. Global analysis of uncapped mRNA changes under drought stress and microRNA-dependent endonucleolytic cleavages in foxtail millet. BMC Plant Biol 15: 241. https://doi.org/10.1186/s12870-015-0632-0

Yu J, Ahmedna M, Goktepe I, 2007. Peanut protein concentrate: Production and functional properties as affected by processing. Food Chem 103: 121-129. https://doi.org/10.1016/j.foodchem.2006.08.012

Zadraznik T, Egge-Jacobsen W, Meglic V, Sustar-Vozlic J, 2017. Proteomic analysis of common bean stem under drought stress using in-gel stable isotope labeling. J Plant Physiol 209: 42-50. https://doi.org/10.1016/j.jplph.2016.10.015

Zhang M, Lv D, Ge P, Bian Y, Chen G, Zhu G, Li X, Yan Y, 2014. Phosphoproteome analysis reveals new drought response and defense mechanisms of seedling leaves in bread wheat (*Triticum aestivum* L.). J Proteomics 109: 290-308. https://doi.org/10.1016/j.jprot.2014.07.010

Zörb C, Schmitt S, Mühling KH, 2010. Proteomic changes in maize roots after short-term adjustment to saline growth conditions. Proteomics 10 (24): 4441. https://doi.org/10.1002/pmic.201000231