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Differentially expressed proteins in sugarcane leaves in response to water deficit stress

Ni-on Ngamhui[1], Chutipong Akkasaeng[1*], Yun J. Zhu[2], Nathpapat Tantisuwichwong[3], Sittiruk Roytrakul[4], and Taksina Sansayawichai[5]

[1]Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand
[2]Hawaii Agriculture Research Center, 94-340 Kunia Road, Waipahu, Hawaii, 96797, USA
[3]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand
[4]Genome Institute, National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Phahonyothin Road, Pathumthani, 12120, Thailand
[5]Khon Kaen Field Crops Research Center, Department of Agriculture, Ministry of Agriculture and Cooperatives, Khon Kaen, 40000, Thailand

***Corresponding author: rut_ak@kku.ac.th**

Abstract

In many areas of the world, water stress is the major constraint limiting the productivity of sugarcane. The objective of this study was to identify proteins that were differentially expressed in sugarcane leaves in response to a water deficit treatment to describe the sugarcane responses at the cellular and molecular levels. Drought-tolerant sugarcane cultivar Khon Kaen 3 stalk cuttings were grown under a controlled environment in a growth chamber where a water deficit treatment was imposed by withholding watering for 5 days. The treatment group continuously received an adequate water supply. Soil moisture content (SMC), leaf water potential (LWP) and relative water content (RWC) were recorded to quantify the water deficit stress. Leaf proteins from non- and water-stressed plants were separated using two-dimensional electrophoresis (2-DE). Image analysis was performed on the electrophoresis gel to locate proteins that were differentially expressed between treatments, which were then identified using liquid chromatography coupled with electrospray ionization ion trap subjected to mass spectrometry/mass spectrometry analysis (LC-ESI-IT-MS/MS) and characterized. Two proteins involved in light-dependent reactions, chlorophyll a-b binding protein 1B-21 and chloroplastic and oxygen-evolving enhancer protein 1, were up-regulated by the stress treatment. Enzymes known to participate in antioxidant networks, including chloroplastic copper-zinc superoxide dismutase (CuZn-SOD), two-cysteine peroxiredoxin (2-Cys Prx) BAS1, superoxide dismutase [manganese] (SOD [Mn]) 3.1, SOD [Mn] 3.4, and isoflavone reductase (IFR) homolog IRL, were also up-regulated. These enzymes all used NADPH as the reducing equivalent, suggesting that linear electron flow (LEF) may dominate the total electron transport activities in sugarcane leaves under water deficit. In addition, two isoforms of ATP synthase beta and ATP synthase alpha subunits were up-regulated under water deficit, indicating that LEF was coupled to the generation of electrochemical gradients across the thylakoid membranes, leading to an increased abundance of ATP synthase beta and ATP synthase alpha subunits. There was increased abundance of a 16.9 kDa class I heat shock protein (HSP) and two isoforms of the elongation factor (EF-Tu) proteins, which are associated with heat tolerance. The identification of proteins regulated by water stress could lead to a better understanding of the cellular response to dehydration, which is an important and fundamental part of improving the stress tolerance of crops.

Keywords: Differentially expressed proteins; Linear electron flow; Sugarcane; Water stress.
Abbreviations: 2-Cys Prx: two-cysteine peroxiredoxin; 2-DE: two-dimensional electrophoresis; APX: ascorbate peroxidase; BSA: bovine serum albumin; CuZn-SOD: copper-zinc superoxide dismutase; CEF: cyclic electron flow; EF-Tu: elongation factor; HSP: heat shock protein; IFR: isoflavone reductase; LC-ESI-IT-MS/MS: liquid chromatography coupled with electrospray ionization ion trap subjected to mass spectrometry/mass spectrometry analysis; LEF: linear electron flow; LWP: leaf water potential; PGR: proton gradient regulation; PSI: photosystem I; PSII: photosystem II; PTOX: plastid terminal oxidase; ROS: reactive oxygen species; RWC: relative water content; SMC: soil moisture content; SOD [Mn]: superoxide dismutase [manganese]; WWC: water-water cycle.

Introduction

Sugarcane is an economically important crop that is widely grown under rain-fed conditions in many countries (Agrawal, 2001; Inman-Bamber, 2004; Laclau and Laclau, 2009). To maximize the crop growth duration and yield of sugarcane in Thailand, the crop is planted after the rainy season in late October to mid-December and harvested in November or later in the next dry season. Sett germination and crop establishment relies on stored soil moisture to support early growth until the onset of the rainy season, which normally begins in May (Matsuo, 2006). The growth and survival of the plants depends on their ability to cope with water deficit stress during the long dry periods. The morphological and physiological acclimation of sugarcane to water stress is well documented (Inman-Bamber and Smith, 2005; Smith et al., 2005; Ming et al., 2006). However, the molecular and cellular responses of the young sugarcane plants to water deficit are not well understood. Water deficit stress invariably causes a decrease in the net photosynthesis of plants (Chaves et al., 2009; Lawlor and Tezara, 2009). In C_3 plants, a reduction in photosynthesis has been attributed to an increase

in stomatal resistance, low expression of ATP synthase, and a limited supply of ATP (Kohzuma et al., 2009; Lawlor and Tezara, 2009). In C_4 plants, however, the stomatal conductance is less affected under drought stress due to the carbon dioxide concentration mechanism (Hatch, 1987). A drought-induced decrease in photosynthesis in C_4 plants may be due to reductions in the activity and quantity of ribulose-1,5-bisphosphate carboxylase oxygenase(RuBisCo) and in the capacity for ribulose-1,5-bisphosphateregeneration (RuBP) (Tezara et al., 2002; Carmo-Silva et al., 2010). A reduction in photosynthetic carbon assimilation under water stress limits the generation of NADPH and ATP. Excessive light energy results in a change in the redox states of photosynthetic cells (Asada, 2006) and an enhanced generation of reactive oxygen species (ROS) (Li et al., 2009). Cellular redox status could be crucial for regulating metabolic processes and responses to abiotic stresses (Meyer and Hell, 2005; Dietz and Pfannschmidt, 2011). The accumulation of ROS to a toxic level provokes cellular damage by initiating oxidative modifications of polyunsaturated fatty acids, DNA, carbohydrates and proteins (Møller et al., 2007). Sugarcane is an NADP-ME type of C_4 plant (Loomis and Amthor, 1999). In photosynthesis in these plants, the LEF for producing NADPH and ATP occurs in the mesophyll cell chloroplasts. This differs from the bundle sheath chloroplasts, which are agranal, and photosystem II (PSII) is depleted as a result of cyclic electron flow (CEF) around photosystem I (PSI) and producing only ATP (Edwards and Walker, 1983). C_4 plants need large amounts of ATP for use in the carbon reduction cycle, carbon-concentration mechanisms and coping with environmental stress (Kramer and Evans, 2011).

Plants have evolved metabolic flexibilities to cope with environmental stimuli. The presence of a malate valve is one of the mechanisms for dissipating excess excitation energy in chloroplasts (Foyer and Shigeoka, 2011). In the malate valve, reducing equivalents are exported as malate to mitochondria for oxidative phosphorylation. Malate valve enzymes may be up-regulated in response to environmental stresses (Scheibe, 2004). The water-water cycle (WWC) is also an alternative sink for excess electrons and a means for the dissipation of excess photon energy. In this cycle, O_2 can be directly reduced by NADPH, which is generated from LEF, to produce O_2^-, which is converted to H_2O_2 by SOD. In chloroplasts, ascorbate peroxidase (APX) catalyzes the reduction of H_2O_2 to H_2O. The operation of the WWC allows LEF to continue, thereby preventing damage to the PSII electron transport components (Ort and Baker, 2002). Chlororespiration and CEF around PSI are involved in balancing the NADPH/ATP budget, preventing the over-reduction of stromal electron acceptors and avoiding photoinhibitory damage to PSII (Rumeau et al., 2007; Shikanai, 2007; Kramer and Evans, 2011). Both chlororespiration and CEF share two electron transport pathways, a proton gradient regulation(PGR)5/PGR1-dependent route also known as the ferredoxin-plastoquinone oxidoreductase-dependent pathway and an NAD(P)H dehydrogenase (NDH)-dependent route. In chlororespiration, electrons from ferredoxin are ultimately delivered to plastid terminal oxidase (PTOX) via plastoquinone, and PTOX reduces O_2 to H_2O. In CEF, however, electrons from plastoquinone are shunted back to PSI via the cytochrome b_6f complex and plastocyanin (Suorsa et al., 2009). Efficient antioxidant systems are required to detoxify ROS that accumulate at a toxic level. The generation of ROS in chloroplasts or mitochondria results in changes in gene expression, suggesting that intracellular communication must occur, but the identities of signaling components remain largely unknown (Apel and Hirt, 2004). Despite their detrimental effects on cellular components and activities, some ROS can also act as signaling molecules in acclimatory responses to environmental stresses (Foyer and Shigeoka, 2011). H_2O_2 is recognized as the most important signaling molecule among ROS (Mubarakshina et al., 2010). H_2O_2 induces the expression of genes encoding proteins with antioxidant functions (Apel ant Hirt, 2004) and elicits the elevated expression of transcripts encoding APX1, catalase 1, iron-SOD and HSP70 (Davletova et al., 2005). To improve crop productivity, it is necessary to understand the mechanisms of plant responses to drought conditions, with the ultimate goal of improving crop performance in the vast areas of the world where rainfall is limiting or unreliable. Consequently, understanding the mechanisms of the responses to cell dehydration is of great importance and is also a fundamental part of improving the stress tolerance of crops. The objective of this study was to identify differentially expressed proteins in sugarcane leaves in response to water deficit stress.

Results

Water supply withholding and water deficits in soil and plants

Withholding the water supply to sugarcane caused reductions in SMC, LWP and RWC, indicating that the sugarcane plant was under water deficit stress. The water stress created by withholding water for 5 days caused a reduction of SMC from 13.2 to 6.2% (Table 1). LWP declined from -0.72 MPa in the plants adequately supplied with water to -2.72 MPa in the water-stressed plants (Table 1). RWC in leaves of sugarcane under water stress was 92.9%, which was lower than the 94.6% that was observed for the well-watered plants (Table 1).

Reproducibility of gels and protein identification

The proteins were well separated into individual spots, and the proteomic maps were similar across all six gels (Supplementary fig. 1). Approximately 515-627 protein spots were resolved in the gels, and 358 matched protein spots were detected in all six gels. However, only 19 proteins could be identified (Fig. 1; Supplementary table 1). The relative abundance of each protein under the non-stressed or stressed condition was indicated by the volume. The mean volumes and standard errors of all the regulated proteins are shown in Fig. 2. Four proteins were down-regulated and 15 were up-regulated in response to water deficit stress (Fig. 1; Supplementary table 1). The magnitudes of changes in protein abundance varied from 1.3- to 3.8-fold (Supplementary table 1). The 2-DE maps indicating the proteins under differential regulation across the six gels are presented in Supplementary fig. 2. The relative changes in these proteins were clearly visualized in three-dimensional views even when they did not display apparent differences in the two-dimensional views (Supplementary figs. 3, 4). Bovine serum albumin (BSA) was identified with a Mascot score of 544, 17 matches and a sequence coverage of 19% (data not shown). Only two replicate protein spots, which gave identical proteins, were obtained. The proteins were classified into three main functional groups: photosynthesis, defense, and miscellaneous. Most proteins in the photosynthesis group were up -regulated, including

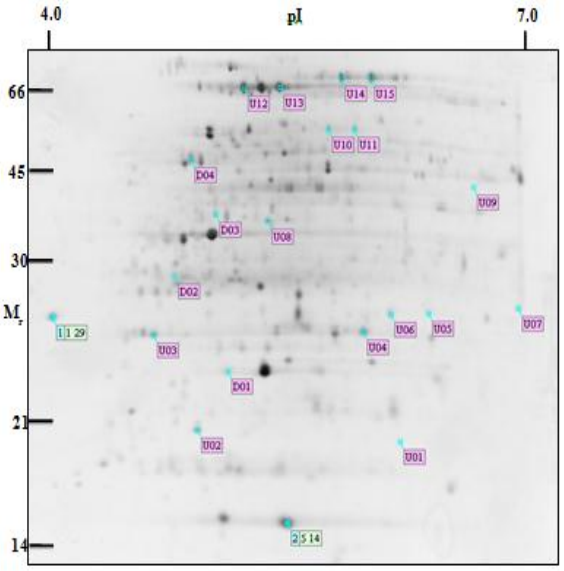

Fig 1. The proteins regulated under water deficit stress. Four proteins were down-regulated (D01-D04), and fifteen proteins were up-regulated (U01-U15). The molecular weight (M_r) and isoelectric point (pI) are indicated.

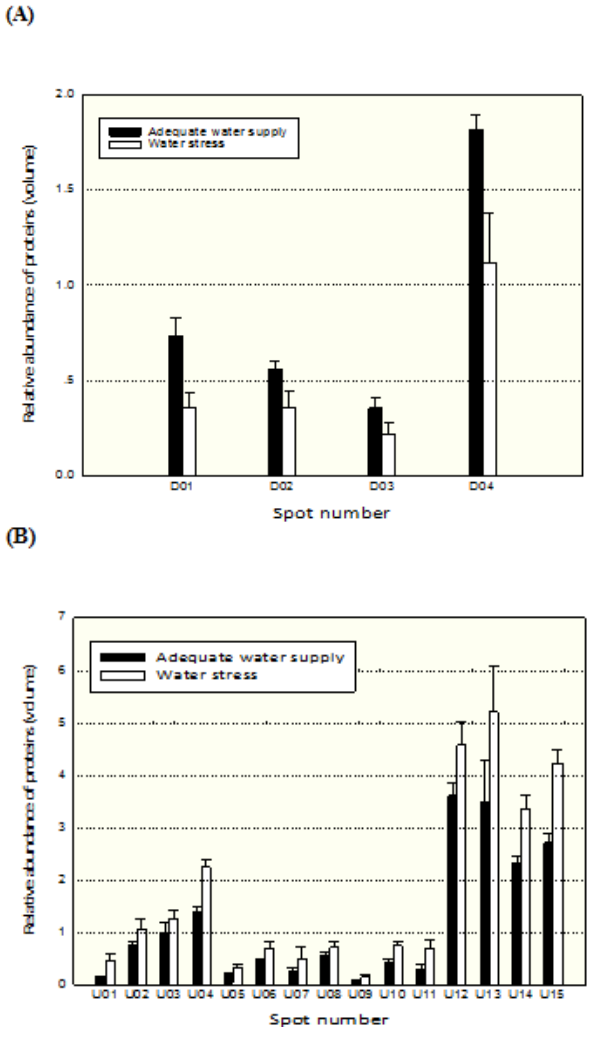

Fig 2. The relative abundance of proteins down-regulated (A) and up-regulated (B) in response to water stress.

chlorophyll a-b binding protein 1B-21, oxygen-evolving enhancer protein 1, and two isoforms of both ATP synthase beta and alpha subunits. Only one protein, the chlorophyll a/b binding apoprotein CP26 precursor, was down-regulated. The defense group contained heat shock protein hsp22; 16.9 kDa class I HSP; chloroplastic CuZn-SOD; SOD [Mn] 3.1, mitochondrial precursor; SOD [Mn] 3.4, mitochondrial precursor; 2-Cys Prx BAS1; two isoforms of EF-Tu; and a 26S proteasome regulatory particle. Only the heat shock protein hsp22 precursor was down-regulated, and the remaining proteins were all up-regulated. Two protein homologs in the last group, ATPase beta subunit and phosphoglycerate kinase, were down-regulated, while the IFR homolog IRL was up-regulated (Supplementary table 1).

Discussion

Multiple pathway protection and oxidative damage

The increases in the abundance of chlorophyll a-b binding protein 1B-21 and oxygen-evolving enhancer protein 1 suggest that LEF was enhanced, leading to the production of ROS at a toxic level. Two enzymes involved in the detoxification of ROS, chloroplastic CuZn-SOD and 2-Cys Prx BAS1, were up-regulated (Supplementary table 1). CuZn-SOD catalyzes the conversion of superoxide anion into H_2O_2 (Asada, 2006), and 2-Cys Prx reduces H_2O_2 to H_2O using NADPH as a source of reducing power (Pulido et al., 2010). The malate valve pathway may be an alternative sink for excess electrons (Niyoki, 2000). Large amounts of malate could be exported to mitochondria for ATP production. However, the mitochondrial electron transport chain may be perturbed under water deficit, resulting in an increased generation of ROS (Pastore et al., 2007). This would activate the antioxidant network to remove ROS. Two isoforms of mitochondrial SOD, SOD [Mn] 3.1 and SOD [Mn] 3.4 (Supplementary table 1), were up-regulated under water deficit stress and may be responsible for scavenging ROS. The relative abundance of the IFR homolog IRL was greater under water deficit (Supplementary table 1). IFR is an NADPH-dependent reductase involved in lignin and isoflavonoid biosynthesis (Shoji et al., 2002). The level of rice IFR-like (*OsIRL*) transcript was induced by H_2O_2. Overexpression of the *OsIRL* gene in transgenic rice plants confers a strong resistance to oxidative stresses (Kim et al., 2010). This suggests that IFR may represent a novel antioxidant system that protects cells against ROS in sugarcane.

Enhanced ATP generation, photophosphorylation and water deficit stress

Dissipation of excitation energy using the reducing power of NADPH coupled to the generation of an electrochemical gradient of protons across the thylakoid membrane or a proton-motive force can be harnessed for ATP synthesis (Kramer and Evans, 2011). Detoxification of ROS using the CuZn-SOD/2-Cys Prx system, the malate valve and IFR may activate LEF and increase the flux of protons into the lumen. In C_3 plants, drought stress leads to an enhanced proton flux, especially through CEF, but the amount and activity of ATP synthase decline (Kohzuma et al., 2009). In C_4 plants, drought conditions cause a lower rate of photosynthetic electron flow rather than increased electron transport to an alternative sink (Ripley et al., 2007), which results in a decreased abundance of ATP synthase subunit alpha and ATP synthase alpha chain (Zhao et al., 2011). In contrast,

wild watermelon from the xerophyte habitat displays a significant increase in the abundance of both ATP synthase alpha chain and ATP synthase beta subunits (Sanda et al., 2011). Our present study clearly supports the results of Sanda and co-workers. Two isoforms of the ATP synthase beta and ATP synthase alpha subunits were up-regulated under water deficit (Supplementary Table 1). Our findings indicate that an enhancement of proton flux into the mitochondrial lumen may be coupled to LEF to supply NADPH for antioxidant networks. This would activate the increased expression and/or the stability of both subunits of ATP synthase, leading to an increased supply of ATP for handling the water stress. Several lines of evidence indicate that CEF contributes substantially to the photosynthetic electron flow to satisfy the cellular demand for ATP under harmful environmental conditions (Kramer et al., 2004; Kramer and Evans, 2011). In NADP-ME-type C_4 plants, the PSII that is involved in LEF may be confined to mesophyll cells (Edwards and Walker, 1983). However, recent evidence indicates that the bundle sheath cell chloroplasts of NADP-ME plants also contain PSII complexes capable of donating electrons to PSI (Romanowska et al., 2008). Because the antioxidant networks mostly use NADPH as reducing equivalent, LEF may be predominant in the total electron transport activities in sugarcane to supply NADPH and ATP for cellular demands in response to water stress.

Water deficit and heat stresses

In the present experiment, sugarcane was grown under high light intensity (720 µmol $m^{-2}s^{-1}$) and high temperature (35°C day/30°C night) to simulate a field environment in a growth chamber. Under these conditions, heat stress may be encountered in addition to the imposed water deficit stress. This phenomenon commonly occurs under field environments (Mittler, 2006). The water deficit resulted in an up-regulation of the 16.9 kDa class I HSP1 and two isoforms of the EF-Tu, with the magnitudes of these changes being 3.8-fold for HSP1 and 1.7- and 2.5-fold for the two isoforms of EF-Tu (Supplementary table 1). During stress, the proteins displayed structural and functional changes. HSP plays important roles in maintaining proteins in their functional conformations, stabilizing and preventing aggregation of proteins and refolding denatured proteins, which are important for cell survival under stress (Wang et al., 2004). EF-Tu plays an important role in polypeptide elongation during protein synthesis. Chloroplast EF-Tu confers heat tolerance by acting as a molecular chaperone that protects chloroplast proteins from thermal aggregation and inactivation (Rao et al., 2004; Ristic et al., 2008). The increased abundance of HSP1 and EF-Tu may provide a means of protecting sugarcane against high temperature stress.

Materials and methods

Plant materials and growth conditions

Drought-tolerant sugarcane (*Saccharum officinarum* complex hybrid cultivar Khon Kaen 3), which is recommended for growth in northeast Thailand, was used in this study. Fresh cane stalks were cut at 10 cm on either side of a node. A single one-eye sett was planted in two plastic pots, with each having a diameter of 28 cm and a depth of 28 cm and containing 13 kg of air-dried soil. Sugarcane was grown under high light intensity and high temperature to simulate field environmental conditions in a growth chamber. Thereafter, the pots were kept in a growth chamber (Model 630, Contherm Scientific Ltd., New Zealand) with an irradiance of 720 µmol$m^{-2}s^{-1}$, a 12-h light/12-h dark cycle, a temperature of 35°C day/30°C night and 85% constant relative humidity.

Water deficit stress treatment and plant samplings

SMC in the pots was maintained at the full water-holding capacity by manual daily watering until the plants had 5-6 fully expanded leaves. The water stress treatment was imposed on a plant in one of the plastic pots by withholding water for 5 days, while SMC in the other pot was maintained at the full water-holding capacity. SMC in the pots was determined using a soil core sampler (Auger set for heterogeneous soils (B08.02), Eijkelkamp, Agrisearch Equipment, The Netherlands). The distal 10 cm of all leaf blades were used for measuring LWP using a pressure bomb (model 3005 HGPL, SoilMoisture Equipment Corp., Santa Barbara, CA, USA). RWC was determined for the second fully expanded leaves of each plant. Leaf blades were cut into 10 pieces of 1 cm length. The fresh weight of the leaf blade cuttings was determined, and then the cuttings were rehydrated at room temperature under dim fluorescent light for 24 h. The turgid weight was recorded, and the cuttings were dried in an oven at 80°C for 48 h. The dry weight of the cuttings was recorded, and RWC was calculated (Barrs and Wealtherly, 1962). The remaining part of first fully expanded leaves from plants in both sets were harvested, frozen in liquid nitrogen and kept at -80°C until protein extraction.

Sugarcane leaf protein preparation

Frozen leaf blades from non-stressed and stressed plants were ground in liquid nitrogen using a mortar and pestle. The total proteins in the ground materials were extracted at 4°C in 2 ml of lysis buffer [7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 60 mM DTT, 2% (v/v) IPG buffer (pH 4-7), 1% (v/v) nuclease mix and 2% (v/v) protease inhibitor mix]. The protein extracts were centrifuged at 13,000 x *g* at 4°C for 20 min, and supernatants were collected. The supernatants were cleaned using a 2-D Clean-Up Kit (GE Healthcare, Little Chalfont, UK), and the protein pellets were resuspended in 100 µl of rehydration buffer [8 M urea, 2% (w/v) CHAPS, 18 mM DTT, 2% (v/v) IPG buffer (pH 4-7) and 0.5% (v/v) protease inhibitor mix]. The protein concentrations were measured using a 2-D Quant Kit (GE Healthcare) (Görg, 2004).

Two-dimensional electrophoresis (2-DE)

One hundred twenty micrograms of protein was resuspended in 250 µl of rehydration buffer. An Immobiline DryStrip (pH 4-7 Linear, 13 cm) was rehydrated using the above protein solution for 12 h at 20°C. During this period, isoelectric focusing was performed at 20°C using a Multiphor II system (GE Healthcare) equipped with a cooling system and 3500XL power supply (GE Healthcare). There were 3 running conditions: 1 min at 300 V, 1 h 30 min at a gradient of 300-3500 V and then 3 h 45 min at 3500 V. Before performing the second dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the focused strips were equilibrated for 15 min in equilibration buffer [6 M urea, 75 mM Tris-HCl (pH 8.8), 2% (w/v) SDS, 30% (v/v) glycerol, 0.002% (w/v) bromophenol blue] containing 1% (w/v) DTT, which was followed by 15 min in the equilibration buffer containing 2.5% (w/v) iodoacetamide. After the equilibration, the strips were transferred onto

12.5%T SDS-polyacrylamide gels (14 cm x 16 cm), and the proteins were resolved in the second dimension at 30 mA/gel at 20°C using a Hoefer SE600 system (GE Healthcare). Proteins from each leaf sample were separated in three replicate gels. Proteins were visualized by silver nitrate staining according to the procedure of Heukeshoven et al. (1986). Two additional 2-D gels were prepared using proteins from non-stressed and stressed plants and stained with Coomassie Brilliant Blue G-250 (GelCode^{TM} blue stain reagent, Pierce Biotechnology, USA) for protein identification by mass spectrometry.

Gel imaging and statistical analysis

Gels were scanned using an ImageScanner equipped with Labscan version 5.0 (GE Healthcare) at 600 dpi. The image analysis of the gels was performed using ImageMaster 2D Platinum 7.0 (GE Healthcare). Four protein spots were used as reference points. The molecular mass (M_r) and isoelectric point (pI) of the reference spots were estimated using molecular mass markers (LMW Calibration Kit, GE Healthcare) as standards. The M_r and pI of all proteins in each gel were estimated by the ImageMaster 2D Platinum 7.0 software. The volume parameter was used to calculate the relative abundance of the proteins. Two sets of gels, one from water-stressed plants and the other from non-stressed plants, were created in a hierarchical match for protein matching and in a hierarchical class for statistical analysis. The protein spots in six gels were matched, and mean volumes of the protein spots under water-stressed and non-stressed conditions were compared. Their statistical differences were calculated using the Student's *t*-test. Up-regulated proteins were indicated when the mean volumes were statistically greater ($p<0.05$) under the stressed condition. Proteins were assigned as down-regulated when their mean volumes were significantly reduced. Two- and three-dimensional views of these proteins were visualized to provide supporting evidence for up- or down-regulated proteins.

In-gel tryptic digestion

To validate the mass spectrometry technique, BSA was separated using SDS-PAGE and stained with Coomassie Brilliant Blue G-250 (Pierce Biotechnology). The BSA strip was excised as plugs from the gel. Up- and down-regulated protein spots were excised from the 2-DE gels. There were two replicate spots for each protein spot analyzed. Proteins were digested in the gel plugs following the methods described by Shevchenko et al. (2006). Briefly, proteins in the gel plugs were reduced with 10 mM DTT in 100 mM ammonium bicarbonate for 1 h at 56°C and alkylated with 100 mM iodoacetamine in 100 mM ammonium bicarbonate for 1 h in the dark at room temperature. The protein in each spot was rehydrated in 20 µl of 12.5 µg ml^{-1} trypsin in 100 mM ammonium bicarbonate for 20 min in an ice bath. In-gel tryptic digestion was performed at 37°C overnight. The resulting peptides were extracted three times with 50 µl of 0.1% (v/v) trifluoroacetic acid and 50% (v/v) of acetonitrile for 20 min each. Pooled extracts were then evaporated to dryness. Dried peptides were dissolved in 3 µl of 0.1% (v/v) trifluoroacetic acid.

Liquid chromatography (LC) coupled with electrospray ionization (ESI) ion trap (IT) subjected to mass spectrometry/mass spectrometry (MS/MS) analysis

Dried peptides were resuspended in 96-well plates for the LC-ESI-IT-MS/MS analysis by adding 8 µl of 0.1% (v/v) trifluoroacetic acid, and they were then transferred into 1.5 ml tubes. Resuspended peptides were centrifuged for 5 min at 10,000 x *g*. Peptide samples were injected into a nanoflow HPLC system (UltiMate 3000 from Dionex Corp., CA, USA) coupled to an HCT ultra IonTrap equipped with a nanospray source (Bruker Daltonics). The chromatographic separations were conducted on reverse-phase (RP) capillary columns (C18; 75 lm i.d.; 15 cm length; LC Packings or Agilent Technologies) with a 200 nL/min flow rate. The electrospray ionization-MS/MS data were acquired using Bruker Daltonics esquire 6.1 in the positive ion mode. The mass data that were collected during the LC-ESI-IT-MS/MS analyses were processed using the software tool DataAnalysis 3.4 Build 169 and converted into mgf files.

Database searching

Mass data in the mgf files were queried using the MASCOT (http://www.matrixscience.com) and OMSSA ms/ms search engines (http://pubchem.ncbi.nlm.nih.gov/omssa/index.htm). In the MASCOT search, the mass tolerance was 0.8 Da for the MS and MS/MS data, which allowed a maximum of 1 missed cleavage by trypsin and the carbamidomethylation of cysteineor oxidation of methionine. The default parameters for mass tolerance were used in the OMSSA search with the carbamidomethylation of cysteine and oxidation of methionine modifications. Only those peptides that were identified by the MASCOT and OMSSA ms/ms search engines were reported.

Conclusion

Sugarcane perceived the water deficit stress and responded with physiological acclimations and changes in protein expression, such as proteins in the photosynthesis pathway and enzymes related to anti-oxidative damage. All these proteins play important roles in protecting cells from damage during rehydration. Thus, proteomics studies could improve our understanding of the plant's response to water stress and could lead to the identification of potential protein markers that are associated with stress tolerance traits, thereby providing the basis for improving the stress tolerance of plants.

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