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RESEARCH ARTICLE

Physiological and Protein Profiling Response to Drought Stress in KS141, a Korean Maize Inbred Line

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Abstract

Understanding the complex response mechanism of a crop to drought is the major step in the developing of tolerant genotypes. In our study, to investigate physiological traits and proteome dynamics, an inbred maize (*Zea mays* L.) line (KS141) was subjected to 10 days of water-withholding at the V5 or V6 leaf stage. The subsequent analysis of their physiological parameters revealed a decreased relative leaf water content, Fv/Fm, stomatal conductance, net CO_2 assimilation rate, leaf transpiration, and water use efficiency, resulting in severe growth retardation of leaf area, stem length and width, aerial part, and root dry matter at 3 and 10 days after withholding water. However, aerial part and root dry matter were little changed during drought stress for 3 days. To understand the proteome dynamics during the 10-day drought stress in maize leaves, comparative proteome analysis was carried out between the well-watered and drought-treated leaves. Proteins were extracted using phenol extraction method from leaves with/without drought stress, and then separated by 2-DE. After 2-DE gel analyses, 14 differentially expressed protein spots were identified by MALDI-TOF mass spectrometry. Out of 14, eleven and three protein spots were found to be up- or down-regulated, respectively. Interestingly, stress-related proteins such as glutathione S-transferase, abscisic stress-ripening proteins, and pathogenesis-related proteins were increased by drought stress. Our study may provide molecular mechanisms and selective markers for drought tolerant maize genotypes.

Key words : Drought, maize, MALDI-TOF, proteome, 2-DE

Introduction

Maize (*Zea mays* L.) is a major staple food for both human consumption and animal feed, as well as a key resource for industrial applications and bio-energy production worldwide. However, drought stress severely affects plant growth and development. In order to survive, plants have adjusted a number of molecular, cellular, physiological, and metabolic responses against drought stress. To date, many studies have attempted to unravel the molecular processes involved in drought tolerance have received much attention (Chaves et al. 2003). Physiological studies have shown that sugars, sugar alcohols, amino acids, and amines function are known to accumulate under drought stress conditions (Seki et al. 2007). Conversely, growth or expansion of aerial parts and root systems were reduced during drought stress. To protect the plant from extensive water loss, reduction in vegetative growth, stomatal closure, and a decrease in the rate of photosynthesis are among the earliest responses to drought (Chaves et al. 2003; Mahajan and Tuteja 2005).

More recently, considerable advances in high-throughput methods such as transcriptome and proteome profiling have enabled researchers to investigate the molecular events involved in plant response to drought on a global scale. Transcriptomic analyses have been used for the large-scale dehydration induced or reduced gene expression and have revealed several genes that are differentially modulated by

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dehydration in maize reproductive and leaf meristem tissue (Kakumanu et al. 2012), and seedling shoots (Zheng et al. 2010). A transcriptomic study of the effect of drought on maize ears and tassels undergoing meiosis revealed raffinose- and trehalose-associated genes as well as other carbohydrate-related processes (Zheng et al. 2004).

To investigate the mechanism of the plant stress response, it is important to use a combination of physiological and biochemical measurements of stress response parameters and to monitor proteins modulated by drought stress. The analysis of the plant proteome offers advantages for the large-scale study of the molecular changes associated with the drought stress response. Proteome analyses have already been carried out evaluating drought-responsive proteins in the important crop species, such as rice (Shu et al. 2010), maize (Benešová et al. 2012), wheat (Peng et al. 2009), and sugar beet (Hajheidari et al. 2005). Several authors also compared the responses of tolerant and sensitive genotypes of maize to drought conditions (de Vienne et al. 1999; Riccardi et al. 2004). Such analyses can be very useful in revealing proteins that are directly involved in the mechanisms underlying plant tolerance to drought.

Nothwithstanding the vast number of studies associated with drought stress, maize cultivars developed by RDA in Korea have been little investigated. The aim of this study was to analyze the differences in physiological and protein expression responses to drought stress of KS141 inbred line developed by RDA in Korea. The KS141 inbred line was subjected to drought stress treatments (withholding water for 10 days) and global protein expression was evaluated using 2-DE analysis combined with MALD-TOF TOF. The results confirm that different physiological responses and different protein expression patterns occur under drought stress, and provide insights as to how changes in protein expression could lead to drought tolerance in maize.

Material and Methods

Plant material and growth conditions

Maize (*Zea mays* L.) plants of KS141, an inbred line, which is a parent of cv. Gangdaok, a Korean elite normal corn F1 hybrid released by the Rural Development Administration (RDA) in South Korea were used. Seeds were planted onto a 1/5000 a Wagner's filled with sandy loam soil on August 1, 2013. Plants were grown in the greenhouse (Suwon, South Korea) and were thinned to a single plant per pot at the V2 leaf stage. At the V6 leaf stage, the water supply was withheld for 10 days. Three days after withholding water, soil moisture content fell to less than 5%. Leaf area, stem length and width, and dry matter of aerial part and root were measured from three or four plants under drought-stressed and well-watered plants at 3 and 10 days after withholding water, respectively.

Determination of leaf chlorophyll

Fifteen fresh maize leaves, of which potable chlorophyll meter (SPAD) values ranged from eight to 59, were harvested and areas of their leaves were measured. The leaves were cut into small pieces, then put into a 15 mL conical tube filled with 5 mL of 95% (v/v) ethanol to extract chlorophyll. The tubes were kept in 4°C for 48 h. Absorbance of each extract was measured at 648 and 664 nm using a spectrophotometer (U-2900, Hitachi High-Technologies Corp., Japan), respectively. Concentration (μg mL^{-1}) of chlorophyll a and b in the extracts was determined using the equations (Miazek and Ledakowicz 2013) as follows:

$$Chl_a = 13.36 \times A_{664} - 5.19 \times A_{648}$$
$$Chl_b = 27.43 \times A_{648} - 8.12 \times A_{664}$$

The leaf chlorophyll content based on leaf area was correlated with the SPAD value (r = 0.97). The standard curve for leaf chlorophyll content was obtained using the SPAD values as follows:

$$Chlorophyll_{a+b}\ (\mu g\ cm^{-2}) = 0.9155 \times \text{SPAD value} - 8.5575 \quad (R^2 = 0.95)$$

The SPAD value of the youngest fully expanded leaf was measured with a chlorophyll meter at 3 and 10 days after withholding water and its chlorophyll content was calculated using the standard curve.

Measurements of gas exchange and chlorophyll fluorescence

Net CO_2 assimilation rate (A), stomatal conductance, and leaf transpiration rate (Tr) were measured on the youngest fully expanded leaf using a portable gas exchange system (Li-6400, LI-COR, Lincoln, NB, USA) at 3 and 10 days after withholding water. A and Tr values were used to calculate the water use efficiency (A/Tr). All other variables within the leaf chamber of the Li-6400 were standardized during measurements; leaf temperature was maintained at 25°C and PPFD at 1,500 μmol quanta m^{-2} s^{-1}. All measurements were replicated three times for each of the three well-watered and drought-stressed plants, respectively. The maximum quantum yield (Fv/Fm) was measured on the youngest fully expanded leaf with a portable chlorophyll fluorometer (PAM 2500, Heinz Walz GmbH, Germany) after at least 30 min of dark adaption using a leaf clip (Dark Leaf Clip LDC-8, Heinz Walz GmbH, Germany). The Fv/Fm was measured for three well-watered and drought-stressed plants, respectively.

Relative leaf water content

A leaf cut was taken from the middle of the youngest fully expanded leaves at 3 and 10 days after withholding water and the fresh weight was determined, followed by floatation on water for up to 48 h. The turgid weight was then recorded, and the leaf cut subsequently oven-dried at about 70°C for 5 days for determination of the dry weight. Relative water content (RWC) of the leaf was calculated as follows (Smart and

Bingham 1974):

$$RWC = \frac{(\text{fresh weiht - weight})}{(\text{turgid weiht - weight})} \times 100$$

Protein extraction

Extraction of proteins was performed as described by Kim et al. (2008). Briefly, fresh maize leaves (KS141 inbred line) were powdered in liquid nitrogen using a pestle and subsequently homogenized with 5 mL of Mg/NP-40 buffer containing 0.5 M Tris-HCl (pH8.3) and 2% β-mercaptoethanol. The mixed samples were centrifuged at 12,000 × g for 10 min at 4°C. The supernatant was mixed thoroughly with an equal volume of Tris-saturated phenol (pH 7.5), following by centrifuging at 12,000 × g for 10 min at 4°C. The phenol phase was mixed with four volumes of methanol containing 0.1 M ammonium acetate, after which protein was precipitated at -20°C for 1 h and then centrifuged at 12,000 × g for 10 min at 4°C. Washing step was conducted according to Kim et al. (2008). Finally, the pellets were rinsed three times with 5 mL of ice-cold acetone, and the washed pellets were stored in 80% acetone at -20°C until the protein content was measured using a 2-D quant kit (GE healthcare, Waukesha, WI, USA).

2-DE and image analysis

2-DE analysis was performed according to Kim et al. (2008). The mixture sample was then centrifuged at 6,500 × g for 15 min at 4°C. The pellets were washed three times with cold methanol, once with ice-cold acetone, and then air-dried. The dried protein pellets were dissolved in rehydration solution (GE Healthcare, Waukesha, WI, USA). The IPG strips (24 cm) were rehydrated in rehydration solution containing equivalent samples (250 µg). IPG focusing was then performed at 50 V for 4 h, 100 V for 1 h, 500 V for 1 h, 1000 V for 1 h, 2000 V for 1 h, 4000 V for 2 h, 8000 V for 5 h, 8000 V for 9 h, and 60 V for 6 h using the IPGphore II platform (GE Healthcare, Waukesha, WI, USA). Each focused IPG strip was then placed into a 20 mL screw-cap tube with 5 mL of equilibration buffer [50 mM Tris-HCl, pH 6.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 100 mM DTT, and 0.1 mg mL^{-1} bromophenol blue]. Strips were then agitated gently at room temperature for 20 min, after which they were secondly equilibrated with 55 mM iodacetamide solution without DTT in equilibration buffer under dark conditions for 20 min with gentle agitation. The 2-DE analysis was carried out on 13% SDS-polyacrylamide gels, after which 2-DE gels were stained by colloidal Coomassie Brilliant Blue (CBB) (Kim et al. 2013). Images were acquired using a transmissive scanner (PowerLook 1120, UMAX) with a 32 bit pixel depth, 300 dpi resolution, and brightness and contrast set to default. Gel spots were automatically detected using the Image Master 2D Platinum software 6.0 (GE Healthcare, Waukesha, WI, USA). The volume of each spot was then normalized as an average of the volume of spots on the gels.

In-gel digestion

Differentially expressed protein spots were subjected to in-gel trypsin digestion according to the method described by Kim et al. (2008). CBB-stained target spots were excised using a razor blade and washed with 50% (v/v) acetonitrile (ACN) in 0.1 M NH_4HCO_3 and vacuum-dried. Dried gels were then treated with 10 mM DTT in 0.1 M NH_4HCO_3 for 45 min at 55°C, after which DTT solution was immediately replaced with 55 mM iodoacetamide in 0.1 M NH_4HCO_3 and samples were incubated for 30 min at room temperature in the dark. Gel pieces were then washed with 50% ACN in 0.1 M NH_4HCO_3 and digested with 12.4 ng μL^{-1} trypsin (Promega, Madison, WI, USA) and 25 mM NH_4HCO_3 in 10 µL of digesting solution overnight at 37°C and air-dried.

MALDI-TOF MS analysis

The matrix solution was prepared by dissolving a-cyano-4-hydroxycinnamic acid (Sigma-Aldrich) in acetone (40 mg mL^{-1}) and nitrocellulose in acetone (20 mg mL^{-1}) (Kim et al. 2013). This solution, the nitrocellulose solution, and isopropanol were mixed 100 : 50 : 50, and 2 µL of the mixture was added to 2 µL of the peptide sample solution. A 1 µL sample of this final solution was spotted immediately onto a matrix-assisted laser desorption/ionization (MALDI) plate and left for 5 min. The MALDI plate was then washed with 0.1% (v/v) TFA. The gel spots were analyzed using a Voyager-DE STR MALDI time-of-flight (TOF) mass spectrometer (PerSeptive Biosystems, Framingham, MA). Parent ion masses were measured in the reflection/delayed extraction mode with an accelerating voltage of 20 kV, a grid voltage of 76.000%, a guide wire voltage of 0.010%, and a delay time of 150 ns. Des-Arg1-bradykinin (m/z 904.4681) and angiotensin 1 (m/z 1296.6853) were used as a two-point internal standard for calibration. Peptides were selected in the mass range of 500 - 3,000 Da. For data processing, the software package PerSeptive-Grams was used. Database searches were performed using Protein Prospector (http://prospector.ucsf.edu) and Mascot (http://www.matrixscience.com) websites.

Results and Discussion

Plant physiological responses to drought stress

The physiological response of the Korean inbred line, KS141, against drought stress was investigated with 25-day-old plants of V6 leaf stage exposed to water-withholding for 10 days under greenhouse conditions. Under drought stress, relative water content (RWC) of the youngest fully expanded leaf of KS141 was significantly reduced by about 16% at 3 days after withholding water (DAW) and about 49% at 10 DAW compared with the well-watered, respectively, indicating the maize plants under water-withholding suffered from drought stress (Fig. 1A). Leaf area, and stem length and width of KS141 were reduced by about 28%, about 57%, and

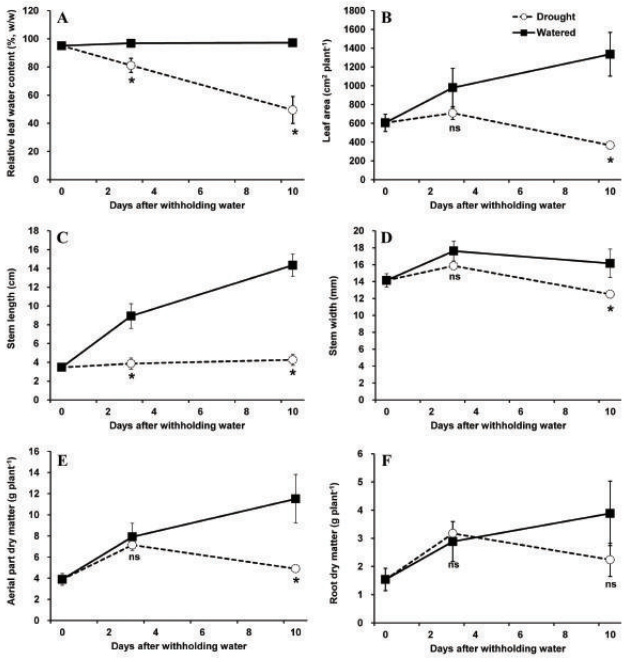

Fig. 1. Relative leaf water content (A), leaf area (B), stem length (C), and width (D), and dry matter of aerial parts (E) and roots (F) of the well-watered and the drought-stressed at 0, 3, and 10 days after withholding water, respectively. Values are means ± standard error (n = 3 or 4). An asterisk indicates than means are significantly different between the well-watered and the drought-stressed, resulted from LSD test (α = 0.05). 'ns' stands for 'not significant'.

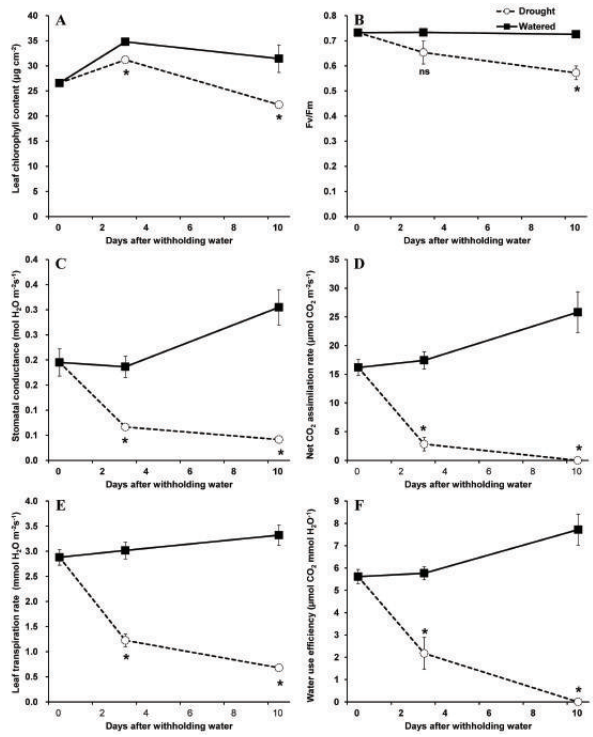

Fig. 2. Leaf chlorophyll content (A), Fv/Fm (B), stomatal conductance (C), net CO2 assimilation rate (D), leaf transpiration rate (E), and water use efficiency (F) of the well-watered and the drought-stressed maize plants at 0, 3, and 10 days after withholding water, respectively. Values are means ± standard error (n = 3 or 4). An asterisk indicates than means are significantly different between the well-watered and the drought-stressed, resulted from LSD test (α = 0.05). 'ns' stands for 'not significant'.

about 10% at 3 DAW, respectively (Figs. 1B - D), showing that stem elongation was rapidly and greatly reduced by drought stress at the V6 leaf stage of KS141. The growth of the leaf area (about 73%), stem length (about 70%), and width (about 23%) were inhibited at 10 DAW, respectively (Figs. 1B - D). Dry matter of aerial parts and roots of KS141 were not hindered at 3 DAW but were greatly restrained at 10 DAW by about 57% and about 42%, respectively (Figs. 1E and F). These results showed that drought stress at the V6 leaf stage of maize may have a severe and rapid effect on leaf growth and stem elongation, resulting in a decrease of dry matter accumulation. These results were consistent with previous research although at different development stages under drought stress (Zheng et al. 2004).

For the youngest fully expanded leaf, physiological parameters such as chlorophyll content, the maximum quantum yield (Fv/Fm) of photosystem II (PSII), stomatal conductance, net CO_2 assimilation rate, leaf transpiration rate, and water use efficiency were determined to measure the effect of drought stress on photosynthesis of the leaf (Fig. 2). Ten and 20% of leaf chlorophyll content were reduced at 3 DAW and at 10 DAW, respectively, and the maximal quantum yield (Fv/Fm) was also 11 and 21% inhibited at three DAW and at 10 DAW, respectively (Figs. 2A and B), indicating that 10 DAW may have an impact on photosynthetic apparatus like chlorophyll content and the maximum quantum yield of PSII. Recently, the ratios of Fv/Fm of six Portuguese maize inbreds ranged from 0.7 to 0.8 under well-watered conditions and decreased under drought stress during the late vegetative stage (Carvalho et al. 2011). Also, drought stress at the early vegetative stage reduced chlorophyll content and the ratio of Fv/Fm in all three different maize hybrids (Efeoglu et al. 2009). Witt et al. (2012) reported a different response of chlorophyll content to drought stress at the flowering stage among maize hybrids in that chlorophyll content was reduced in all hybrids except one (Witt et al. 2012). Sixty-four percent of stomatal conductance, 84% of net CO_2 assimilation rate, 59% of leaf transpiration rate, and 62% of water use efficiency were inhibited by drought stress at 3 DAW (Figs. 2C - F). These data suggested that KS141 has a quick response to drought stress closing stomata to prevent leaves from losing water resulting in reduction of net CO_2 assimilation subsequently leading to a reduction in dry matter accumulation, leaf growth, and stem elongation.

The comparative 2-DE analysis of maize leaves under drought stress

The proteome procedure between well-watered and drought-stressed leaves is commonly used according to Kim et al. (2013). Separation of proteins by 2-DE is advantageous as it gives an overview of protein profiling modulated by various stresses. We extracted proteins from KS141 leaves grown in pairs (well-watered vs. drought stressed) for 10 days using phenol extraction with neutral IPG strips (pH 4 - 7) to obtain the best resolution of proteins on 2-DE gels.

Table 1. Proteins identified by MALDI-TOF/TOF.

Spot No.	Accession No.	Putative Function	Score	Expect	MP	SC (%)[1]	Mr(kD)/pI[2]	Organism
1	gi|226506958	Peptidyl-prolyl cis-trans isomerase	100	0.0035	22	40	46.8/4.91	*Zea mays*
2	gi|4582787	Adenosine kinase	157	6.7e-009	17	37	36.5/5.23	*Zea mays*
3	gi|226505740	DIMBOA UDP-glucosyltransferase BX9	196	8.5e-013	23	40	50.3/5.15	*Zea mays*
4	gi|297818282	Predicted protein	60	31	9	14	19.3/8.89	*Arabidopsis*
5	gi|194688752	Unknown	184	1.3e-011	15	27	47.7/5.95	*Zea mays*
6	gi|195634659	Fructose-bisphosphatealdolase	215	1.1e-014	16	28	41.8/7.63	*Zea mays*
7	gi|414869036	TPA: Putative methionine synthase family protein	190	3.4e-012	28	35	86.8/5.49	*Zea mays*
8	gi|307697215	ATP synthase CF1 alpha subunit	403	1.7e-033	29	43	55.7/5.73	*Chasmanthium latifolium*
9	gi|413950795	Hypothetical protein ZEAMMB73_038317	138	5.4e-007	18	29	46.5/6.11	*Zea mays*
10	gi|113207534	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	179	4.3e-011	25	36	50.4/6.46	*Medemia argun*
11	gi|195639070	Lactoylglutathionelyase	192	2.1e-012	20	53	35.3/6.62	*Zea mays*
12	gi|168489	Glutathione S-transferase I	152	2.1e-008	12	32	24.0/5.44	*Zea mays*
13	gi|226493193	Abscisic stress ripening protein 2	205	1.1e-013	15	85	14.9/6.15	*Zea mays*
14	gi|195615416	Pathogenesis-related protein 1	88	0.051	10	65	17.1/5.38	*Zea mays*

[1] SC: sequence coverage.
[2] Mr/pI: Theoretical molecular weight/isoelectric point

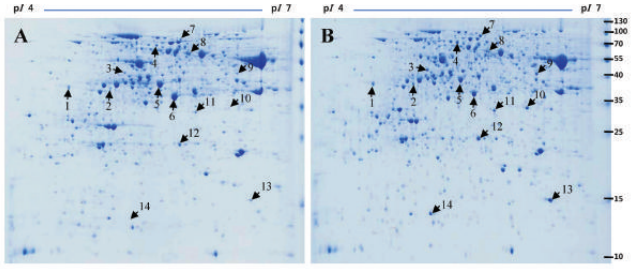

Fig. 3. Representative 2-DE gel of Korean maize inbred line (KS141). (A) Well-watered condition. (B) 10 days drought stress condition. Distinct protein spots detected on the 2-DE gel are marked by arrows. A total of 500 μg protein was used for each 2-DE gel.

From the comparison of 2-DE protein spots, we identified 14 differentially expressed protein spots between well-watered and drought-stressed KS141 leaves using the ImageMaster software (Figs. 3 and 4). Eleven protein spots were markedly increased or newly induced (spots 1 - 4, 7, and 9 - 29) under the drought stress conditions, whereas spots 5, 6, and 8 were decreased (Figs. 4 and 5). These results suggest that drought stress caused the up- or down-regulation of a few proteins in maize leaves, perhaps in response to plant adaptations to drought stress.

Identification of proteins involved in drought-stressed KS141 leaf

To further understand the mechanism of drought stress in maize, we identified differentially expressed proteins by MALDI–TOF using database searches with Protein Prospector and Mascot. The identified proteins were classified based on functional categories established by Schnable et al. (2009). These proteins which responded to drought stress were found to be involved in diverse biological processes, covering three stress-related proteins, four metabolism related proteins, two photosynthesis related proteins, one protein folding/proteolysis related protein, and three unknown proteins (Table 1). The highly induced proteins in response to drought stress compared to well-watered maize leaves were attributed to stress-related proteins including glutathione S-transferase I (GST, spot 12), abscisic stress ripening protein 2 (ASR2, spot 13), and pathogenesis-related protein 1 (PR-1, spot 14). Induction and accumulation of PR proteins in response to various abiotic stresses have been documented in maize (Pechanova et al. 2013). The expression of GSTs can be induced by a range of abiotic stresses, such as drought, salt, and cold (Gallé et al. 2009). Currently, the activities of PR proteins in response to drought stress were increased in white clover leaves (Lee et al. 2008). ZmPR-10 showed significant sequence homology to proteins from the PR-10 family, including one from white lupin that has been shown to possess RNase activity (Bantignies et al. 2000). Expression levels of a number of ASR genes are rapidly increased in response to water deficit, cold, salt, and limited light (Kalifa et al. 2004), the cloning of maize ASR as genes linked to drought resistance (de Vienne et al. 1999). From our experiments, we found that most of stress-related

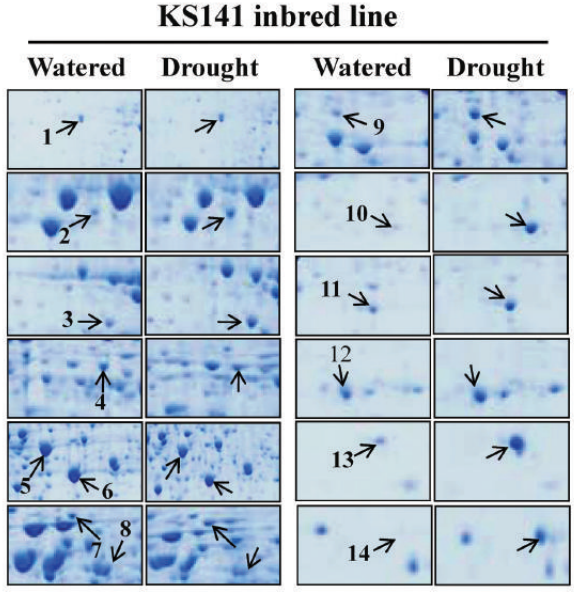

Fig. 4. Expression profiles of protein spots in well-watered and drought-stressed KS141 inbred lines. A close-up view of differentially expressed protein spots was generated.

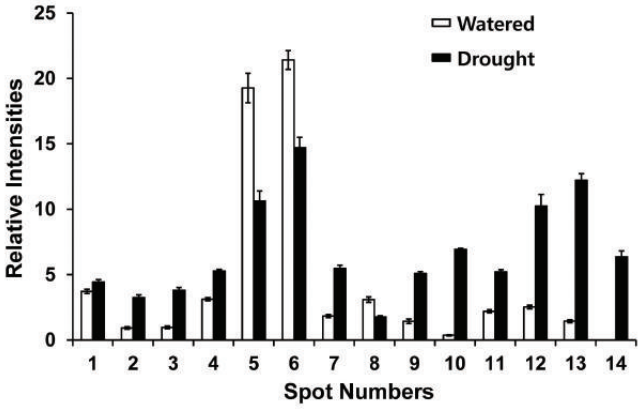

Fig. 5. The relative intensities of each spot were measured using ImageMaster software, and the bar graph was generated based on the average intensity of three gel replicates.

proteins were up-regulated by drought stress, which implies that these stress-related proteins may be important in plant tolerance to drought stress.

Four enzymes related to metabolism responded to drought stress in the leaves of the KS141 inbred maize line. Adenosine kinase (ADK, spot 2), UDP-glucosyltransferase (UGT, spot 3), and lactoylglutathione lyase (LGL, spot 11) were increased, whereas fructose-bisphosphate aldolase (FBA, spot 6) was decreased by drought stress. ADK is a housekeeping enzyme that catalyzes the phosphorylation of adenosine (Ado) into adenosine monophosphates (AMP) (Moffatt et al. 2000). ADK was found to be up-regulated, possibly due to an O2- and ATP-increased availability under drought stress. Previous research found that FBA mRNA levels increased in response to high salinity, drought, and/or ABA in maize (Hu et al. 2012), and *Arabidopsis thaliana* (Lu et al. 2012) but the identified FBA in this study was decreased by drought stress. To our knowledge, little prior functional studies found a drought effect upon metabolism related to proteins including UGT and LGL. Further research is required to determine the roles of these metabolism-related proteins in the drought response in Korean maize inbred lines.

Finally, three proteins changed under drought conditions were associated with photosynthesis and protein modification. Levels of ATP synthase CF1 alpha subunit (spot 8) and ribulose bisphosphate carboxylase/oxygenase activase (RCA, spot 10) were lower or higher under drought stress than in well-watered conditions, respectively. ATP synthase has been implicated to play an indirect role on ion homeostasis under salt stress (Gao et al. 2011). The decreased ATP production via down-regulation of ATP synthase CF1 subunit is attributed to decreased photosynthetic rates in stressed plants (Caruso et al. 2008, 2009). RCA is a molecular chaperone that is involved in switching Rubisco from an inactive to an active conformation (Spreitzer and Salvucci 2002), and the up-regulation of this protein may act to relieve the damage to Rubisco caused by drought stress (Ji et al. 2012). Another protein involved in protein folding was peptidyl–prolyl cis-trans isomerase (PPIase, spot 1). In our study, PPIase had been increased by drought stress in KS141. A similar abundance profile was observed in the study of peptidyl–prolyl cis-trans isomerase activity in cultivars of sorghum under drought (Sharma and Singh 2003).

Conclusion

In this study, Korean maize inbred line (KS141) plants that were subjected to water-withholding at the V6 leaf stage showed rapid inhibition in stem elongation and had about 73% less leaf area than the well-watered. Water-withholding made plants quickly close leaf stomata leading to no CO_2 assimilation and caused about 29 and about 21% decrease in leaf chlorophyll content and the maximum quantum yield compared to the well-watered plants. These plant growth inhibition and photosynthesis inactivation mainly due to stomatal closure by drought stress results in dry matter accumulation of aerial parts and roots. Three expression stress-related proteins such as GST, ASR, and PR were mainly modulated by drought stress. Although the obtained information is a step forward in understanding the underlying adaptation mechanism of maize to drought stress in Korea, this physiological analysis combined with a proteomic approach will be useful for understanding molecular physiological processes against drought stress and give clues for finding drought tolerant maize lines and genes related to drought tolerance

Acknowledgements

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