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Proteomics analysis of the photo-oxidation mutant 812HS rice (*Oryza sativa* L.) exposed to high light intensity

Chunfang Lv[1], Jing Ma[1†], Ze Yuan[1], Yuwen Wang[1], Guoxiang Chen[1], Zhiping Gao[1]*, Chuangen Lv[2]*

[1]School of Life Sciences, Nanjing Normal University, Nanjing 210023, China
[2]Institute of Food and Crops, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

***Corresponding author: E-mail: jiayoubwg@sina.cn**
[†]The author contributed equally to the paper.

Abstract

The 812HS rice line, a mutant of two-line sterile rice, exhibits leaf photo-oxidation during the tillering-jointing stage since its chloroplasts are susceptible to damage induced by strong sunlight. To determine the proteomic response to light intensity, the 812HS rice line was exposed to natural light and shade (about one fourth natural light), and two-dimensional electrophoresis in combination with matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry was used to compare the proteomic expressions in the rice leaves. Although over 1000 reproducible proteins were detected, only 9.61 % of them displayed differential expression with 1.5-fold abundance. A search of the National Center for Biotechnology Information database revealed 53 proteins, 34 down-regulated and 19 up-regulated under highlight. Among these identified proteins, six ones were related to disease and defense, implying the defense and protection mechanism was built under strong light stress. The increase in S-adenosylmethionine synthetase involved in the biosynthesis of the phytohormone ethylene might contribute to the phenotypic modulation from green to etiolation in photo-oxidation-sensitive leaves. These results suggest the existence of a complex regulatory mechanism of the proteomes in photo-oxidation-sensitive 812HS rice under light stress.

Keywords: 812HS rice (*O*ryza sativa L.); Light intensity; Photooxidation; Proteomic; Two-dimensional electrophoresis.
Abbreviations: ACN_acetonitrile; ATP_adenosine triphosphate; CBB_coomassie brilliant blue; DTT_dithiothreitol; GAPDH_glyceraldehyde 3-phosphate dehydrogenase; IEF_isoelectric focusing; MALDI-TOF/TOF_matrix-assisted laser desorption/ionization time-of-flight/time-of-flight; OEC_oxygen-evolving complex protein; RCA_ribulose-1,5-bisphosphate carboxylase/oxygenase activase; RLS_rubisco large subunit; ROS_reactive oxygen species; TFA_trifluoroacetic acid; 2-DE-two dimensional electrophoresis.

Introduction

Rice (*Oryza sativa* L.) is not only a critically important food crop plant (Sasaki and Burr, 2000) but also an excellent model plant of cereal crops in scientific research. In the past few decades, studies of its physiology, ecology, genetic background, and molecular constitution have been performed (Komatsu et al., 2003). With the completion of the rice genome sequencing project, “rice proteomics” has entered the central stage of rice biology (Yu et al. 2002; Goff et al., 2002). Rice proteomics studies have focused on response to diverse abiotic (drought, salt) and biotic stresses, which provide fundamental insight into function of individual proteins along with their dynamic interaction network (Sun et al., 2014). Among the abiotic factors, light intensity and temperature can be considered to be the most serious factors limiting plant growth and yield (Foyer, 2002; Reddy et al., 2004). Kirchhoff found that sunlight intensity causes irreversible damage to photosynthetic apparatus in thylakoid membranes under high light conditions (Kirchhoff, 2014).

Light plays an essential role in plant photosynthesis, and the photosynthetic rate gradually increases as light intensity is enhanced. When the light energy absorbed by plants exceeds the capacity of light utilization in photosynthesis, photo-oxidation occurs (Reddy and Raghavendra, 2006). Photo-oxidation, one of the most deleterious phenotypic traits, can reduce photosynthetic productivity by >10% (Ögren and Rosenqvist, 1992). Photo-oxidative stress in plants can cause the accumulation of reactive oxygen species (ROS), resulting in intracellular membrane lipid peroxidation and chlorophyll degradation (Ji et al., 2002), especially under photo-chilling, salinity, and drought stresses (Asada, 1999; Foyer and Noctor, 2000; Reddy et al., 2004). Numerous studies indicating multiplex protective mechanisms against the deleterious effects of photo-oxidative stress in photosynthetic apparatuses such as the xanthophyll cycle (Demmig-Adams and Adams, 1996), cellular antioxidant system (Matsubara et al., 2005), and photorespiration (Iacono and Sommer, 1996) were performed under high light irradiance. A comparative study of photo-oxidative damage differences was conducted in japonica and indica rice (*Oryza sativa*) under photo-oxidation and shading conditions. The study showed that the photo-oxidation-sensitive cultivar was easily harmed when exposed to photo-oxidation (Li and Jiao, 2000).

The photo-oxidation mutant 812HS rice line was derived from a segregation population of the Indica photothermo-sensitive male sterile line 812S. The 812HS rice line showed the same agronomic traits as 812S in the seedling stage; however, the leaves of the 812HS line turned yellow from the top by strong sunlight when the flag leave

was spread, a phenomenon that was not noted in the 812S line (Lai et al., 2012). Genetic analysis and gene mapping showed that photo-oxidation in the 812HS line was controlled by a new dominant leaf photo-oxidation 1 gene (*LPO1(t)*), which was preliminarily mapped between RM307 and RM401 on a segment of chromosome 4 (Lai et al., 2012). The mutant provides very novel research material for further studies on the regulatory mechanism and associated gene function of photo-oxidation (Xia and Lv, 2012).

Most of the work to date on the physiological mechanisms of photo-oxidation in rice has been done under artificial photoinhibition conditions (Law et al., 2001). However, little is known about which proteins are responsive to photo-oxidation conditions in the 812HS line. In the present study, the proteome profile of 812HS flag leaves were analyzed using two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight/ time-of-flight (MALDI-TOF/TOF) mass spectrometer by shading, which could provide theoretical information for cultivating superior rice breeds in the future.

Results and Discussion

Comparison and functional classification of proteome map in 812HS rice

Proteomics analysis is an effective method for investigating the integral changes in the gene expression of plants under environmental stresses (Yan et al., 2006; Lee et al., 2009). To identify the differential expressions of proteins in rice treated with different light intensities, 2-DE was performed of the proteins extracted from the flag leaves. After CBB R-250 staining and software analysis, general proteome patterns under pH 4-7 were built for rice leaves (Fig. 1). A comparative analysis was conducted on these 2-DE gels using Image Master 2D Platinum software. A 1.5-fold quantitative change was set as the criterion, and 53 protein spots were differentially displayed in the 812HS rice line between the control and natural light treatment conditions (Table 1). Using the proteomic method, Yang et al. (2007) found some differential proteins of de-etiolated rice seedling responses to light stress and constructed the light-responsive network at the protein level.

The 53 identified proteins of diverse expression patterns could be divided into seven categories by function (Bevan et al. 1998) (Table 2). The most abundant category was energy-related proteins, while those involved in signal transduction, protein destination and storage, and secondary metabolism were seen at the same abundance in the identified proteins. The other functional categories included defense and unclear classification.

Proteins involved in energy production

The present study detected enhanced expression of glycine-rich proteins on signal transduction and identified some new light-responsive proteins including S-adenosylmethionine synthetase and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). These changes provided new insight into the protein responses of plants to light exposure. In our study, a total of 53 differentially accumulated protein spots were analyzed and identified by MALDI-TOF/TOF mass spectrometry (Table 1). The most abundant identified proteins on our gels were involved in energy production. Among them, 17 were involved in energy production, including three related to light reactions of photosynthesis and 12 were associated with the carbon assimilation cycle as well as inorganic pyrophosphatase and nicotinamide adenine dinucleotide phosphate–dependent mannose 6-phosphate reductase. Adenosine triphosphate (ATP) synthetase F0 (spot no. 279) and oxygen-evolving complex protein1 (OEC) (spot nos. 112, 192) related to the light reactions of photosynthesis were down-regulated in the 812HS line compared with rice under shade condition. ATP synthetase F0 is an integral membrane enzyme that couples the free energy of proton transfer down an electrochemical gradient to the synthesis of ATP and the universal energy currency of all organisms (Yang et al., 2007). OEC is directly involved in water oxidation, located on the luminal side of photosystem II, and formed by several extrinsic proteins and a manganese cluster (Mn_4Ca) (Zaharieva et al., 2011). Their down-regulation may have been attributed to a decrease in photosynthetic capacity in rice under high light stress.

The proteins involved in the photosynthetic carbon assimilation cycle

The proteins involved in the photosynthetic carbon assimilation cycle were all decreased except for ribulose-1,5-bisphosphate carboxylase/oxygenase activase (RCA), the key enzyme for the rapid formation of critical carbamate in the active site of the Rubisco activase protein. It is modulated either by a reaction with CO_2 and Mg^{2+} to carbanylate, a lysine residue in the catalytic site, or by the binding of inhibitors within the catalytic site (Portis, 2003). Our result showed that RCA (spot no. 256) was strongly up-regulated in most expanded leaves of rice under stress. High light stress can reduce the photosynthesis either by impairing the Rubisco activation state, which is often attributed to RCA activity loss, or by reducing the abundance of Rubisco proteins. Thus, the up-regulated RCA could protect the photosynthesis machinery under high light stress. Despite similar protection from the RCA, different protective strategies were adopted by rice in different conditions. For instance, iorganic pyrophosphatase (spot no. 15) might contribute to improving protection against stress by increasing abundance. The spot no. 15 enzyme, on the other hand, plays an important role in sucrose synthesis (Kornberg, 1962), which synthesized polysaccharides from sucrose to enhance the plant's resistance to high light intensity (Aguilera et al., 1999).

Spots (245, 249, 360, 364, and 280) were identified as the same protein, Rubisco large subunit (RLS). However, they were located at different positions on the gels, with similar Mr and pI, indicating that they might be RLS isoforms. It can be assumed that the RLS is up-regulated under stress since its expression is enhanced. Spots no. 228, 244, 251, 286 and 336 were identified as ribulose-1,5-bisphosphate carboxylase large china precursor with similar Mr and pI, which were down-regulated similar to ribulose-1,5-bisphosphate carboxylase (spot no. 14). A recent report showed that the sharp reductions in major leaf photosynthetic proteins, including the abundantly present ribulose-1,5-bisphosphate carboxylase/oxygenase, had occurred in the ozone condition. Ozone, a highly notorious environmental pollutant, has a damaging effect on rice seedlings at the proteome level (Agrawal et al., 2002). Thus, these results indicated that the decline in photosynthetic protein expression might be correlated with light and other stresses.

Disease/defense proteins are also important for stress response

In current experiment, hairpin binding protein 1 (spot no. 92),

Table1. Protein identification through MALDI-TOF-MS.

	Accession no.	Description/ Function	Score	SC (%)	Theoretical Mr	Theoretical pI
Decreased proteins						
1[6]	gi|11466848	Photosystem I subunit VII	257	43	9406	6.51
3[7]	gi|115465862	Os06g0101600	177	15	15624	5.61
12[7]	gi|115434488	Os01g0144100	227	11	20676	6.71
19[7]	gi|115456241	Os03g0820600	160	30	16050	5.72
21[5]	gi|2331133	Glycine-rich protein	39	14	16074	7.82
40[7]	gi|115477166	Os08g0504500	290	23	25552	6.85
43[7]	gi|115484359	Os11g0167800	260	18	15456	6.2
47[6]	gi|108862278	Thylakoid lumenal 29.8 kDa protein, putative, expressed	325	30	19218	6.75
64[3]	gi|51090747	Chaperonin 21 precursor, putative	231	20	24835	7.68
66[2]	gi|11177845	Glutathione S-transferase OsGSTF3, putative	46	4	25117	5.81
81[7]	gi|115474285	Os07g0694700	435	22	27215	5.21
88[7]	gi|54290425	Unknown protein	232	11	34622	9.11
97[7]	gi|115460338	Os04g0602100	315	12	38443	8.67
120[7]	gi|115478314	Os09g0277800	71	33	39167	9.1
124[2]	gi|20286	Peroxidase	167	11	33311	5.77
126[7]	gi|115434036	Os01g0106400	298	23	33481	5.69
245[1]	gi|11466795	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	288	12	53418	6.22
256[1]	gi|1778414	Ribulose-1,5-bisphosphate carboxylase/oxygenase activase	277	8	48055	5.85
279[1]	gi|194033257	ATP synthase F0 subunit 1	165	47	55624	5.85
Increased proteins						
2[7]	gi|115465862	Os06g0101600	176	15	15624	5.61
7[7]	gi|38344542	OSJNBa0027P08.10	172	23	16673	9.37
8[7]	gi|115441831	Os01g0916600	272	23	15537	7.98
14[1]	gi|671740	Ribulose-bisphosphate carboxylase	221	31	15111	6.59
39[7]	gi|115474635	Os08g0129200	355	31	19024	6.28
55[7]	gi|115478330	Os09g0279500	186	10	26779	8.53
57[7]	gi|125527970	Hypothetical protein OsI_04011	310	19	21749	5.86
92[2]	gi|38679325	Harpin binding protein 1	207	9	28457	8.92
96[7]	gi|115453797	Os03g0565200	130	11	34138	8.36
99[7]	gi|115459134	Os04g0490800	138	9	39811	6.75
112[1]	gi|739292	Oxygen-evolving complex protein 1	72	8	26603	5.13
123[1]	gi|41053022	NADPH-dependent mannose 6-phosphate reductase, putative	162	9	35730	5.88
133[7]	gi|115448263	Os02g0713400	73	12	34940	6.19
172[2]	gi|108705994	Glyceraldehyde-3-phosphate dehydrogenase B, chloroplast precursor, putative, expressed	261	11	34024	4.99
187[6]	gi|22748337	Gln1-orysa glutamine synthetease root isozyme, putative	115	11	38779	5.73
192[1]	gi|739292	Oxygen-evolving complex protein 1	221	12	26603	5.13
210[7]	gi|115477843	Os08g0562100	396	11	47492	6.96
217[4]	gi|3024122	S-adenosylmethionine synthase 2	336	11	43330	5.68
225[7]	gi|116317759	OSIGBa0127D24.2	186	46	52221	8.39
228[1]	gi|108862318	Ribulose bisphosphate carboxylase large chain precursor, putative	250	14	56547	9.04
244[1]	gi|108862318	Ribulose bisphosphate carboxylase large chain precursor, putative	110	10	56547	9.04
246[1]	gi|89280711	ATP synthase F0 subunit 1	173	4	55532	5.85
249[1]	gi|11466795	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	356	9	53418	6.22
251[1]	gi|108862318	Ribulose bisphosphate carboxylase large chain precursor, putative	286	14	56547	9.04
277[7]	gi|115436320	Os01g0328700	266	14	53009	7.21
278[7]	gi|115436320	Os01g0328700	149	52	53009	7.21
280[1]	gi|57283874	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial	114	51	53331	6.23
286[1]	gi|108862318	Ribulose bisphosphate carboxylase large chain precursor,	252	6	56547	9.04

		putative				
310[7]	gi|38344034	OJ991214_12.15	259	22	18271	4.67
311[2]	gi|149390991	2-cys peroxiredoxin bas1	301	44	15404	5.13
320[7]	gi|115448263	Os02g0713400	187	10	34940	6.19
336[1]	gi|108862318	Ribulose bisphosphate carboxylase large chain precursor, putative	252	6	56547	9.07
343[2]	gi|75114857	ATP-dependent zinc metalloprotease FTSH 2	297	6	72607	5.54
360[1]	gi|11466795	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	393	10	53418	6.22
364[1]	gi|11466795	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	191	6	53418	6.22

a)SC is the abbreviation for sequence coverage. b) MW and pI are theoretical. c) Superscripts of the protein numbers show the functional categories to which the proteins belong. 1 stands for energy production; and 2, 3, 4, 5, 6 and 7 stand for the categories of disease and defense, protein destination and storage, secondary metabolism, signal transduction, unclear classification and unknown function proteins, respectively.

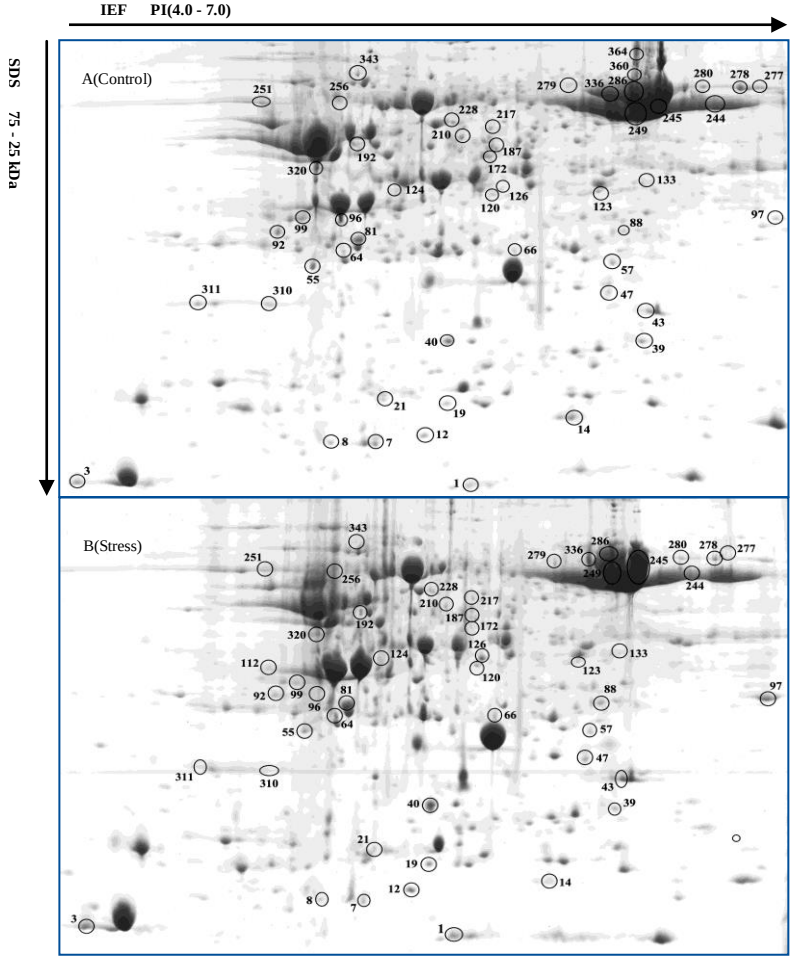

Fig 1. Protein analysis of 812HS rice leaves with control (A) and sunlight (B) in 812HS rice leaves. The differentially accumulated proteins between control and sunlight exclusion are indicated by circles and standard spot numbers on a representative gel. See Table 1 for a detailed list of proteins.

glyceraldehyde-3-phosphate dehydrogenase B (spot no. 172), ATP-dependent zinc metalloprotease FTSH2 (spot no. 343), and enzyme 2-cys peroxiredoxin bas1 (spot no. 311) were down-regulated, while glutathione S-transferase OsGSTF3 (66) and peroxidase (spot no. 124) and enzyme 2-cys peroxiredoxin bas1 (spot no. 311) were up-regulated. GAPDH is a central glycolytic protein with critical role in energy production and an abundant and key enzyme in glycolysis and gluconeogenesis in most plants (Sirover, 1999). Moreover, GAPDH is a protein with multiple functions involving the translational control of gene expression (Mezquita et al., 1998). For the last decade, there were many reports that GAPDH works as a stressor associated with oxidative stress in cells that undergo apoptosis (Saunders et al., 1997). It was also reported that GAPDH expression is naturally enhanced in Leishmania spp, which are naturally resistant to nitric oxide. The decreased expression of GAPDH may be a sign that the protective system response to light in the 812HS line was disintegrating. The hairpin protein group, which was first found and identified by Wei et al. (1992) in Erwinia amylovora, may result in multiple plant responses which have beneficial effects on crop improvement (Alfano and Collmer, 2004). The current results showed the possible defense mechanism

Table 2. Classes and variation tendency of proteins identified on 2-D gels.

Category	Spot number	Up(+)/down(−)
Energy production		
ATP synthase F0 subunit 1	279	−
Oxygen-evolving complex protein 1	112,192	−
Ribulose-1,5-bisphosphate carboxylase/oxygenase activase	256	+
Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	245,249,280,360,364	−
Ribulose-bisphosphate carboxylase	14	−
Ribulose bisphosphate carboxylase large chain precursor, putative	228,244,251,286,336	−
Inorganic pyrophosphatase	70	−
NADPH-dependent mannose 6-phosphate reductase, putative	123	−
Disease and defense		
2-cys peroxiredoxin bas1	311	−
Peroxidase	124	+
Glutathione S-transferase OsGSTF3, putative	66	+
ATP-dependent zinc metalloprotease FTSH 2	343	−
Glyceraldehyde-3-phosphate dehydrogenase B, chloroplast precursor, putative, expressed	172	−
Harpin binding protein 1	92	−
Protein destination and storage		
Chaperonin 21 precursor putative	64	+
Secondary metabolism		
S-adenosylmethionine synthase 2	217	−
Signal transduction		
Glycine-rich protein	21	+
Unclear classification		
Photosystem I subunit VII	1	+
Thylakoid lumenal 29.8 kDa protein, putative, expressed	47	+
Gln1-orysa glutamine synthetase root isozyme, putative	187	−

a) The "+" represents the up-regulated protein spot on the 2-D gels. b) The "−" represents the down-regulated protein spot on the 2-D gels.

defense mechanism of rice plants in response to light by inducing hairpin proteins, thus enhancing photosynthesis and acclimatization. The significantly up-regulated glutathione S-transferase OsGSTF3 as well as down-regulated hairpin binding protein 1 was found in highly light-stressed 812HS line, which protects cells from injury induced by a wide range of stresses in plants (Marrs, 1996). Furthermore, the overexpression of the glutathione S-transferase gene improves the tolerance of Arabidopsis to salinity and oxidative stresses (Sharma, 2014). In addition, ROS generation is usually increased when the stresses are treated. ROS is mostly generated by the absorption of excess excitation energy leading to the over-reduction of the electron transport chains in plants subjected to photo-oxidative stress (Reddy and Raghavendra, 2006). Simultaneously, non-enzymatic and enzymatic systems might contribute to enhancing plant resistance . In our study, 2-cys peroxiredoxin bas1 (spot no. 311) and peroxidase (spot no. 124), which effectively repaired ROS-injured tissue, were up-regulated. Our proteomics analysis was consistent with that induced by peroxidases, and 2-cysteine peroxiredoxins were deemed crucial in controlling chain-type reactions following the initiation of lipid peroxidation in plant cell membranes (Baier and Dietz, 1999; Mullineaux and Karpinski, 2002). ATP-dependent zinc metalloprotease, which is mainly located in the thylakoid membrane, contributes to membrane protein regulation (Paulo et al., 2003). The filamentation temperature sensitive H protein precursor and other molecular chaperonin and enzymes correlated with proteometabolism were found in rice seeding responses to cold stress (Mann et al., 2000). In our study, the different changes in proteins might contribute to improving the stronger light resistance in the response of rice to environmental stresses and alleviating the damage induced by stresses (Nopvím et al., 2004).

Proteins related to signal transduction

On signal transduction, the abundance of glycine-rich protein (spot no. 21) in rice under light stress is higher than that of rice exposed to shade, which is attributed to extracellular signaling transduction, damage repair, RNA shearing, ripening, and gene expression (Ringli et al., 2001). We found that it plays important roles in stress resistance, and their accumulation could alleviate the damage induced in plants under stresses. In addition, S-adenosylmethionine synthetase, which is related to phytohormone synthesis, was increased under shade conditions. S-adenosylmethionine synthetase, which catalyzes the biosynthesis of S-adenosylmethionine from l-methionine and ATP, acts as a precursor molecule in the biosynthesis of the phytohormone ethylene (Yang and Hoffman, 1984). Research has shown that the accumulation of S-adenosylmethionine synthetase induced by salt stress may play a role in adaptation to salt stress (Espartero et al., 1994).

Materials and Methods

Rice material

The 812HS rice line (*Oryza sativa* L. indica cultivar) was

grown in pots under natural light (PFD 2500-3000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and shade (PFD 600-800 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The flag leaves were spread and collected (Xu et al., 2016). The samples were immediately frozen in liquid nitrogen and kept at -80°C until the protein extraction step (Yang et al., 2006).

Protein extraction

Protein extraction was performed according to Wang et al. (2006) by phenol extraction methanol/ammonium acetate precipitation.

Two-dimensional electrophoresis

The 2-DE was performed according to Carpentier et al. (2005) with some alterations. The sample was diluted with isoelectric focusing (IEF) rehydration buffer (8 M urea, 2% 3-[(3-cholamidopropyl) dimethylammonio]-1 -propanesulfonate, 50 mM dithiothreitol (DTT), 0.2% Bio-Lyte 3/10 ampholyte, 0.001% bromophenol blue). Sample strips 24 cm long with pH 4-7 (Bio-Rad, Bio-Science) were rehydrated in 450 mL rehydration buffer containing approximately 1,000 mg of proteins. The IEF was performed on an Ettan IPGphor II (GE Healthcare) at 2 h at 1000 V, 2 h at 500 V, 3 h at 1,000 V, 5 h at 8,000 V (gradient), and 80,000 Vh at 8,000 V. After the first-dimensional run, gels were incubated for 15 min in 10 mL of equilibration buffer (6 M urea, 30% glycerol, 2% SDS, 0.002% bromophenol blue, 75 mM Tris; pH 8.8) containing 1% w/v DTT and subsequently for 15 min in 10 mL equilibration buffer including 2.5% w/v iodoacetamide. The 2-DE was performed on the Ettan DALTsix Electrophoresis System (GE Healthcare) with lab-cast 1.0 mm SDS polyacrylamide gels (12.5%). After the electrophoresis, each gel was stained in 250 mL of Coomassie brilliant blue (CBB) solution containing 100 mL of methanol, 25 mL of acetic acid, and 0.25 g of CBB R-250.

Protein visualization, image analysis and spot identification

The stained gels were scanned at a 300 dpi resolution by an ImageScanner III (GE Healthcare) and the spot detection and gel comparison were performed using an Image Master™ 2D Platinum version 7.0 software. Protein digestion and MALDI-TOF/TOF analyses were performed as described by Ning (2013) with light modification. First, the protein spots were individually excised from the gels. Each small gel piece with protein was destained with a solution containing 100 mM NH_4HCO_3 and 30% v/v acetonitrile (ACN) until each gel piece was colorless thoroughly. After being vacuum-dried, the gel pieces were rehydrated with 10 ng/μL trypsin (Promega, Madison, WI, USA) in 25 mM NH_4HCO_3 at 4°C for 1 h and then subjected to in-gel digestion in the same solution 37°C overnight. After digestion, the protein peptides were collected and the gels were washed with 0.1% trifluoroacetic acid (TFA) in 60% ACN to collect the remaining peptides. The peptides were re-dissolved in one volume of saturated α-cyano-4-hydroxycinnamic acid in 50% v/v ACN containing 0.1% TFA. The resulting peptide mixture of each protein was analyzed by Ultraflex II MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Bremen, Germany). The peptide mass fingerprint data of each sample were acquired using Mascot software (http://www.matrixscience.com). Hits were considered significant according to the Mascot score ($p<0.05$).

Conclusions

In summary, our initial proteomic investigation reveals a complex cellular network of exposure to light in the 812HS rice line. The network consists of a broad range of metabolic processes, including energy production, disease/defense, protein destination and storage, secondary metabolism, signal transduction, and other unknown and unclassified proteins. The presence of these proteins with no clear functions indicated there is much to be learned about the molecular and proteomic events caused by sunlight stress. A future study on a comparative genome transcriptome analysis may be required, and whether the observed protein variations reflect changes in gene expression or some enzyme activities requires further confirmation.

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