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ORIGINAL RESEARCH

A Proteomic Analysis of Leaf Responses to Enhanced Ultraviolet-B Radiation in Two Rice (*Oryza sativa* L.) Cultivars Differing in UV Sensitivity

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Abstract To determine the proteomic response to UV irradiation, two cultivars, i.e., Lemont (UV tolerant) and Dular (UV sensitive), were exposed to natural and enhanced ultraviolet-B (UV-B) irradiation for 1, 7, and 14 days, and two-dimensional gel electrophoresis in combination with mass spectrometry (MS) and bioinformatics were used to compare the different proteomic responses in the leaves of the two cultivars. Thirty-nine proteins were up- or downregulated following the UV-B treatments. Among them, 30 increased or decreased more than 1.5-fold in abundance. They were further tested by using matrix-assisted laser desorption/ionization time of flight MS and performed a database search. Twenty-four proteins were thus identified. These identified proteins were mostly upregulated in Lemont, whereas only 14 of them upregulated in Dular. Nine proteins involved in glycometabolism and fatty acid metabolisms, signal transduction, and protein synthesis and folding in Dular were not changed. These results suggest that there was a complex regulative mechanism on the proteomes in rice leaves upon UV-B exposure.

Keywords Mass spectrometry · *Oryza sativa* L. · Proteome · Ultraviolet-B radiation

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Introduction

Depletion of stratospheric ozone (O_3) caused by anthropogenic chlorofluorocarbons has increased the amount of UV radiation, especially UV-B (280–315 nm) that reaches the earth's surface (Ganguly et al. 2009). For every percent decrease in O_3, the amount of biologically effective UV-B radiation is predicted to increase by approximately 2% (Scotto et al. 1988). Because the DNA and proteins in the plants can absorb UV spectrum, they are easily damaged. The results may include aberrant variations of morphology and genetic substances, damage of photosynthetic pigments, and/or decline of photosynthetic capacity of the plants. In turn, they indirectly cause unfavorable impacts on human existence and development. Therefore, the effects of UV-B on crops and the plant's protective mechanisms against the radiation have been studied throughout the world since the 1970s. However, most of the reports focused on soybean (*Glycine max* (L.) Merr) (Peng and Zhou 2009; Liang et al. 2006), wheat (*Triticum aestivum* L.) (Agrawal and Rathore 2007; Correia et al. 1999; Li et al. 1998), barley (*Hordeum vulgare* L.) (Schmitz-Hoerner and Weissenböck 2003; Fedina et al. 2003), and pea (*Pisum sativum* L.) (Qu et al. 2006; Mackerness et al. 1999). It was in the 1990s when the international projects under the direction of the American EPA and Philippine IRRI systematically studied rice in regard to the effects and mechanisms brought about by the radiation and global climate change (Olszyk and Ingram 1991).

Rice is considered a useful monocotyledonous plant model for genetic and molecular study (Khan and Komatsu 2004). Over the years, most literature relating to the effects of enhanced UV-B radiation on rice has been focused on three areas (Teramura et al. 1991; Dai et al. 1992; Barnes et al.

1993; Sato et al. 1994, 2003; Lin et al. 1999; Wu et al. 2007; Cassi-Lit et al. 1997; Markham et al. 1998; Masakazu et al. 2001; Xu and Qiu 2007): (1) morphological characteristics and ultrastructure, (2) physiochemical mechanism, and (3) inheritance of resistance. And they studied mostly based on a single rice cultivar, and only a few compared the UV-B sensitivity between two or more cultivars.

Recently, the proteomic approach to reveal the mechanisms of plants under stress has become popular. Proteomic analysis provides information on functional genomics, including identification of open reading frames from genome sequences, protein subcellular localization, to novel components involved in the biogenesis (Zivy and Vienne 2000).

Reports of proteomic analysis of rice grown under stress conditions, such as drought (Ke et al. 2009; Salekdeh et al. 2002), ozone (Feng et al. 2008), heavy metal (Lee et al. 2009), chilling (Lee et al. 2010), and pathogen (Yu et al. 2008), as well as maize (Casati et al. 2005) and soybean (Xu et al. 2008) grown under enhanced UV-B radiation, are found. However, none was on rice under enhanced UV-B exposure. In this study, leaf proteomes were determined for two different UV-B-sensitive rice cultivars, Lemont (tolerant) and Dular (sensitive). The cultivars' responses to UV-B stress at the proteome level were detected to reveal the mechanism of rice sensitivity to the irradiation, which could provide theoretical information for cultivating superior rice breeds in the future.

Materials and Methods

Plant Materials

Two varieties of rice (*Oryza sativa* L.) were used in the experiment: Lemont (UV-B tolerant, USA) and Dular (UV-B sensitive, India) (Markham et al. 1998; Fang et al. 2009).

Growth Conditions and Treatments

Seeds were surface-sterilized with 5% (*w*/*v*) sodium hypochlorite, soaked in distilled water for 2 days, transferred to wet tissue paper, germinated on Petri dishes at 30° C, and sown in seedling plate. At the three-leaf stage, uniform seedlings were transplanted into small holes in plastic plates, and the plates were floated in a pot (50 cm× 35 cm×15 cm) with 10 l Kimura's B complete nutrient solution. During the hydroponics, the solution was maintained at pH 5.5 at all times and replaced with new one every 7 days.

The UV-B radiation was supplied by fluorescent lamps (Beijing Electric Light Sources Research Institute). The lamps were wrapped with a 0.1-mm cellulose diacetate film (West Design Product Co., Ltd, UK) to block off light with wavelengths below 280 nm (UV-C), and hung above the rice plants. The height of the plant canopy was adjusted to maintain 30 cm below the lamps for a constant UV-B exposure. The rice seedlings were allowed to grow for 2 weeks under enhanced UV-B radiation. The UV-B radiation was applied at the top of the leaf canopy for a period of 7 h per day (from 09:00 to 16:00 h) at an intensity of 18.6 kJ m^{-2} day^{-1} (It equals to the enhance dose of UV-B radiation when 25% depletion of the stratospheric 0_3 layer occurred in the summer solstice in Fuzhou). Rice under solar radiation was taken as control. The second uppermost leaf of the treated rice plant as well as the control was collected on day 1, 7, and 14 after the light exposure. The samples were immediately frozen in liquid nitrogen and stored at −80°C prior to protein analysis.

Protein Sample Preparation

Approximately 5 g of leaf tissues was homogenized in a mortar with 0.5 g polyvinylpyrrolidone (PVP) followed by immersing the mixture in liquid nitrogen. The fine, frozen powder was then suspended in a precooled 10% TCA in acetone solution containing 0.07% β-mercaptoethanol for protein extraction overnight at −20°C. After centrifuged at 18,000*g* for 25 min at 4°C, the supernatant was discarded and the precipitate resuspended with the same solution as used previously at −20°C for 1 h before repeating the centrifugation under the same conditions. The extraction and centrifugation were duplicated until a clear supernatant was obtained. The final precipitate was dried under vacuum. The dried material was then dissolved in a lysis buffer containing 8 mol L^{-1} urea, 4% CHAPS, 40 mmol L^{-1} Tris, and 65 mmol L^{-1} DTT, with sonication for 20–25 min, and followed by centrifugation at 18,000*g* for 15 min. The supernatant was subjected to two-dimensional gel electrophoresis (2-DE). The protein loading was normalized using the Bradford assay (Bradford 1976).

2-DE and Gel Imaging

The 2-DE was performed under the following conditions. The lab-made strip was 18 cm long and loaded with 160 μg of sample. For isoelectric focusing, the voltage was increased from 200 to 300, 400, 500, and 600 V for 30 min, to 800 V for 14 h, and to 1,000 V for 4 h. Prior to the electrophoresis in the second dimension, the strips were incubated for 30 min in an equilibration buffer (60 m mol L^{-1} Tris–HCl at pH 6.8 containing 2% SDS, 5% β-mercaptoethanol, 10% glycerol, and 0.05% bromophenol blue). The strips were then inserted onto SDS–PAGE gels, sealed with agarose containing 1% bromophenol blue, and

electrophoresed. The 2-DE gels were run at 10°C for 20 mA per gel, followed by silver staining performed as described by Vorum et al. (1999).

Image Analysis

After stained, the 2-DE images were obtained with an image scanner and analyzed with the Imagemaster 2D Elite 5.0. Protein spots were selected when a significant expression variation of 1.5-fold or more existed as compared with the control.

In-Gel Enzymatic Digestion of Proteins

Gel pieces containing the selected protein spots were washed twice with 50% acetonitrile containing 50 mmol L^{-1} ammonium hydrogen carbonate (NH_4HCO_3) for 20 min and drained off the solvent each time. The gel pieces were further immerged in 100% acetonitrile for 10 min drained and dried at 37°C in an oven for 5 to 10 min. The dried gels were rehydrated with 12.5 ng μL^{-1} trypsin at 4°C for 30 min in a refrigerator followed by ion overnight incubation at 37°C. A solution containing 50% acetonitrile and 0.1% trifluoroacetic acid (TFA) was added subsequently, and allowed to stand for 40 min. Tryptic peptides were moved to a 96-hole plate and dried with nitrogen flow. The peptides were redissolved in 0.7 μl 0.5 g L^{-1} α-cyano-4-hydroxy cinnamic acid (CHCA) containing 0.1% TFA and 50% acrylonitrile (ACN). And the mixture was spotted onto a stainless-steel MALDI target plate for protein analysis.

MALDI-TOF/MS Analysis

Protein analysis was performed on a 4700 Proteomics Analyzer (TOF/TOFTM) (Applied Biosystems, USA) at the Proteomics Research Center of Fudan University, China. Peptides were evaporated with a Nd:YAG laser at 355 nm and accelerated with a 20-kV injection pulse for the time-of-flight analysis. The data were collected with positive ion mode and the automatic metadata acquiring system. The mass scan range of the peptide mass fingerprinting (PMF) was 700–3,500 Da. Five ion peaks with large intensity were used for the tandem mass spectrometry. The spectra were corrected using myoglobin enzymatic digestions as external standards.

Database Search and Protein Identification

The search program, GPS (Applied Biosystems, USA)-MASCOT (Matrix Science, London, UK), was used for the database search. The parameters applied included: database= NCBI nr; species genome=*O. sativa* (rice); search method= combined; allowing maximum digestion leakage=site 1; and enzyme=trypsin. The mass error range was PMF 100 ppm, MS/MS 0.6 Da.

Results

Construction and Analysis of Proteins with Differential Expressions in Rice Cultivars of Different Resistance to UV-B Exposure

The 2-DE was used to identify spots modulated by UV-B radiation on Lemont and Dular rice. Each 2-DE gel had about 800 protein spots covering range of pH 3.0–10.0 and molecular weight 14–116 kDa, according to their isoelectric points. Thirty-nine protein spots, labeled 1 to 39, exhibited significant changes in the two rice accessions in comparison with their respective control. An example of the images is shown in Fig. 1, which represents the 2-DE gel of Lemont exposed to the enhanced UV-B radiation for 14 days. By comparing the gels produced in three treatment stages, the 31 protein spots for the UV-B-tolerant cultivar, Lemont, displayed significant changes in expression. These spots were No. 1–28, 30, 31, and 37. Among them, No. 2, 4, 5, 6, 9, 10, 11, 13, 14, 15, 16, 17, 20, 21, 23, 24, 25, 26, 27, 28, 30, 31, and 37 were identified successfully (Table 1). But under the same conditions, only 22 protein spots (i.e., No. 6, 9, 11, 14, 16, and 23–39) for the UV-B-sensitive cultivar, Dular, had significant changes in expression. Among them, 15 spots, i.e., No. 6, 9, 11, 14, 16, 23, 24, 25, 26, 27, 28, 29, 30, 31, and 37, could be clearly identified (Table 1). Partial comparisons between the images of some differentially expressed proteins in the leaves of the two rice accessions in the control (CK) and the treatment (TR) groups with different lengths of UV-B treatment time are shown in Figs. 2 and 3. Hence, 30 protein spots with good reproducibility and more than 1.5-fold changes in abundance were subjected to MALDI-TOF/MS. Out of them, 24 proteins were identified using database research.

Function Classification of Protein Spots

Functions of the 24 proteins were identified on the website Mascot (http://mascot.proteomics.com.cn/) according to their PMFs. Among them, No. 16 and 29 were the same protein, an example shown in Fig. 4. The results indicated that these proteins with differential expressions were involved in photosynthesis, carbohydrate metabolism, protein metabolism, fatty acid metabolism, signal transduction, cell defense, etc. It suggested that the rice responded to the enhanced UV-B radiation in a multifaceted manner.

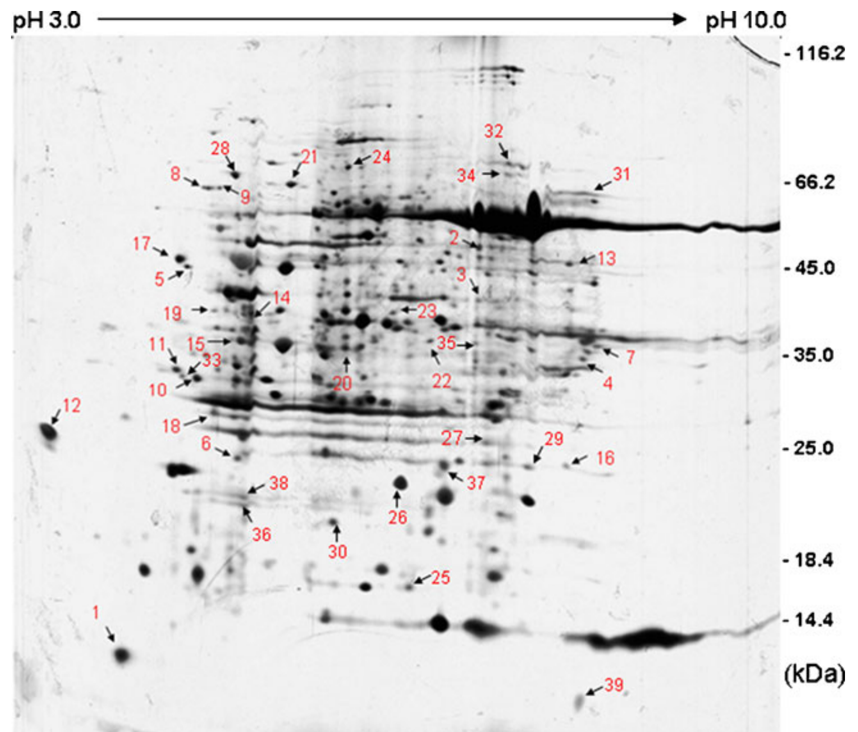

Fig. 1 2-DE image of leaf proteins in Lemont under UV-B stress for 14 days

Results of the function classification are shown in Table 2. Most of the identified proteins increased in the UV-B-tolerant rice, Lemont, especially those involved in protein metabolism, fatty acid metabolism, signal transduction and cell defense. Only spots No. 16 (i.e., carbonic anhydrase, which is related to photosynthesis) and No. 31 (i.e., dihydrolipoyl dehydrogenase, which is associated with carbohydrate metabolism) decreased after 14 days of the UV-B treatment. On the other hand, the 15 spots detected for the UV-B-sensitive rice, Dular, showed different patterns during the treatment stages, with only seven spots changed (4 increased and 3 decreased) after the 14-day UV-B treatment. It revealed that the same metabolic pathways were altered in different rice cultivars exposed to the enhanced UV-B radiation, and that the diverse expression patterns and different proteins involved might be due to the length of the treatment as well as the differences in the specific proteins' sensitivity to UV-B.

Intracellular Locations of Differentially Expressed Proteins

The result of the intracellular localization by WoLF PSORT (http://wolfpsort.seq.cbrc.jp) for rice (*O. sativa* L. ssp *japonica*) is shown in Fig. 5. The statistics analysis demonstrated that most of the identified proteins were found within the cell, except one on the cell wall, with mostly in chloroplast (47.8%), mitochondrion and cytoplasm, and some in membrane structures including endoplasmic reticulum, Golgi membrane, and tonoplast.

Discussion

In this study, 30 proteins in the rice, especially the tolerant-type, were found to be significantly affected by UV-B stress, largely upregulation expressed. Similar results were reported on maize (Casati et al. 2005), in which 178 maize leaf protein spots were altered by UV-B radiation, and most of them increased. In other studies, Kaspar et al. (2010) used 2-DE and label-free LC-MS/MS^E approaches to determine the barley seedling leaf responding to UV-B radiation, which showed that the main reaction in epidermis tissue covering primarily initial stress responses, such as the accumulation of antioxidants for detoxification of generated reactive oxygen species (ROS) as well as induction of stress-responsive proteins and proteins of primary metabolism for supply of precursors for secondary product formation, i.e., flavonoids. The similar results were found in the transcriptome study of UV-B radiation effects on the leaf transcriptome of grapevine (*Vitis vinifera* cv. Malbec) plantlets (Pontin et al. 2010) and the other plants (Jansen et al. 1998; Frohnmeyer and Staiger 2003). It was reported that photosynthetic capacity of plants decreases when they were exposed to UV, including degradation of photosystem II D1 and D2 proteins, reduction of activity and amount of Rubisco, damage of thylakoid membrane, destruction of chlorophyll and carotinoides, etc. (Jansen et al. 1998; Frohnmeyer and Staiger 2003). The study on soybean (Xu et al. 2008) showed that 67 peptide spots were changed and the total number of spots increased in abundance, with a

Table 1 Differentially expressed proteins in rice leaves under UV-B stress identified by MALDI-TOF/MS

Spot no.	Protein identification	MOWSE score	Accession no.	Mr.(D)	PI	Sequence coverage
2	Aminotransferase y4uB	95	Q7XN11	56439	6.33	9%
4	Putative delta 12 oleic acid desaturase	46	Q8GVJ4	44952	9.29	22%
5	Vacuolar ATPase B subunit	30	Q7FV25	54025	5.07	13%
6	Plastid-specific 30S ribosomal protein 2	89	Q6H443	26665	8.53	27%
9	Putative 4-coumarate coenzyme A ligase	25	Q6ETN3	59603	5.04	14%
10	Putative ribose-5-phosphate isomerase	39	Q6ZEZ2	26956	5.25	9%
11	Putative fruit protein PKIWI502	196	Q6Z6B5	30677	5.44	23%
13	Glutamate dehydrogenase	117	Q852M0	44314	6.15	14%
14	Putative tyrosine phosphatase	131	Q9LKK3	27229	6.73	19%
15	Putative inorganic pyrophosphatase	126	Q6ZGJ8	31762	5.80	19%
16	Carbonic anhydrase	178	O80422	29099	8.41	31%
17	Putative Peptidyl-prolyl cis-trans isomerase	40	Q6YW78	46453	4.82	23%
20	Oxygen-evolving complex protein 1	105	Q7M1Y7	26489	5.13	28%
21	Glutamine synthetase	126	Q0J9E0	46613	5.96	6%
23	L-threonine 3-dehydrogenase (Quinone oxidoreductase)	175	Q7EYM8	39558	7.63	25%
24	Putative phosphoglycerate mutase	169	Q5QMK7	60752	5.42	30%
25	Peroxiredoxin-2C	188	Q9FR35	17280	5.58	51%
26	Glycine-rich protein	37	O22385	16017	7.82	34%
27	GSH-dependent dehydroascorbate reductase 1	358	Q9MB31	23541	5.65	50%
28	RuBisCO subunit binding-protein alpha subunit	158	Q2QU06	61093	5.12	11%
29	Carbonic anhydrase	158	O80422	29099	8.41	40%
30	Rubisco large subunit	94	Q37247	45102	8.43	5%
31	Dihydrolipoyl dehydrogenase	360	Q10FN0	52610	7.21	31%
37	Superoxide dismutase [Mn]	91	Q0DJ64	24982	6.50	11%

comparable number of spots that decreased. The different results obtained with soybeans might be due to the experimental design. In the experiment with soybeans, sunlight exposure was used as the treatment to compare with a UV-B-blocked light exposure. Furthermore, unlike this study, there was no supplementary UV-B applied for comparison in that study. Singh et al. (2010) found that supplement of UV-B stress caused severe damage to the proteome of test plant, and the reduced proteins are related to primary metabolism, structural integration, and photosynthetic protein, but the defense/stress-related protein was induced in test plant.

Plant species and even genotypes within species can differ greatly in their responses to UV-B radiation (Li et al. 2000). Bassmam et al. (2002) reported increased net photosynthesis under moderate UV-B irradiance in *Pseudotsuga menziesii*. Kumari et al. (2009) showed that the photosynthetic rate of Acorus plants was increased in low UV-B treatment, which led to higher accumulation of biomass. He et al. (1994) found that UV-B induced *O. sativa* L. cv. Lemont producing more water-soluble proteins that may be related to its greater UV-B tolerance. These responses occurred without apparent deleterious effects on the photosynthetic properties of rice leaves. In contrast, the same hardening doses caused damage to pea plants, as indicated by decreases in photosynthetic quantum yield, maximum photosynthetic capacity, and chlorophyll content per unit leaf area, as well as water-soluble and total protein contents. In this study, more protein spots in Lemont responded to the enhanced UV-B treatment than in Dular. It indicated that more positive reactions happened in the tolerant rice cultivar in the process of detecting, preventing, and/or repairing the damage by UV-B. The proteins involved in photosynthesis were all increased, except carbonic anhydrase (spot No. 16), in Lemont under UV-B stress. The RuBisCO binding-protein alpha subunit (spot No. 28) was upregulated in the three treatment stages, and the abundance expressed of RuBisCO large subunit (spot No. 30) and the oxygen evolution complex protein 1 (spot No. 20) increased after the 14-d UV-B treatment. It was postulated that the content of the polypeptide oxygen evolution complex protein increased, affecting the photosynthesis process and accelerating the synthesis of RuBisCO alpha subunit binding-protein by natural adaptation. All proteins associated with photosynthetic process, such as oxygen evolution complex protein 1, RuBisCO

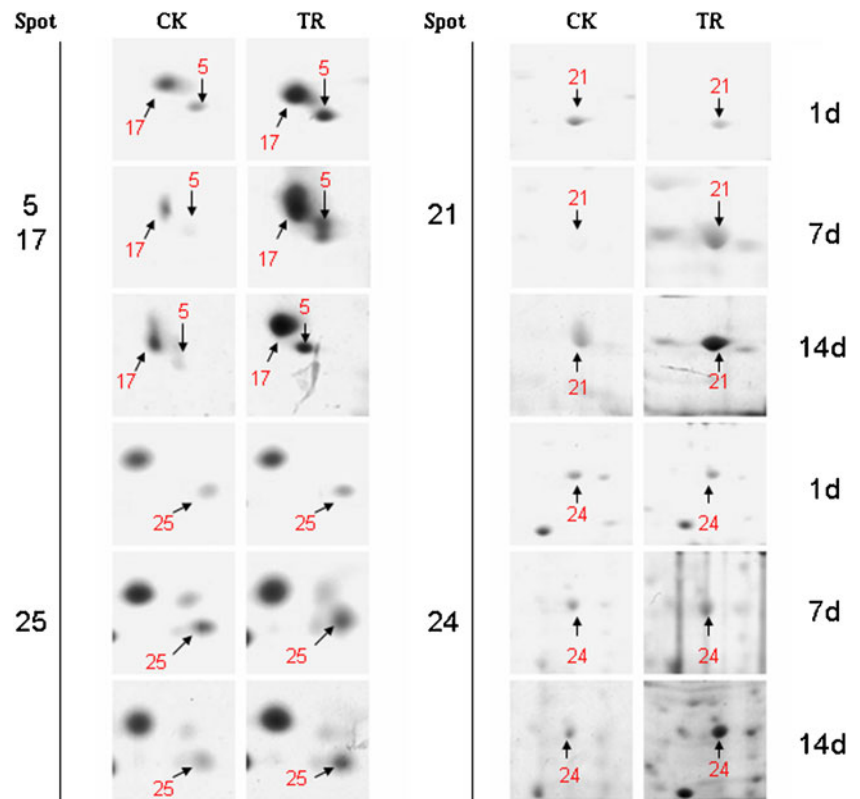

Fig. 2 Partial comparisons between images of some differentially expressed proteins of Lemont leaves in CK and TR groups at three treatment stages. *CK* control, *TR* treatment. *1d*, *7d*, and *14d* indicate the days of the enhanced UV-B radiation treatment; *dash* no differential expression, *upward arrow* expression upregulation, *downward arrow* expression downregulation. (Same for the remaining figures and tables)

large subunit, RuBisCO alpha subunit binding-protein, and carbonic anhydrase (spots No. 29 and 16), were altered. After 7-day UV-B exposure, spots No. 28 and 30 in Dular were upregulated, but did not change after 14-day treatment. It suggested that Dular was more sensitive and vulnerable to UV-B than Lemont. Carbonic anhydrase,

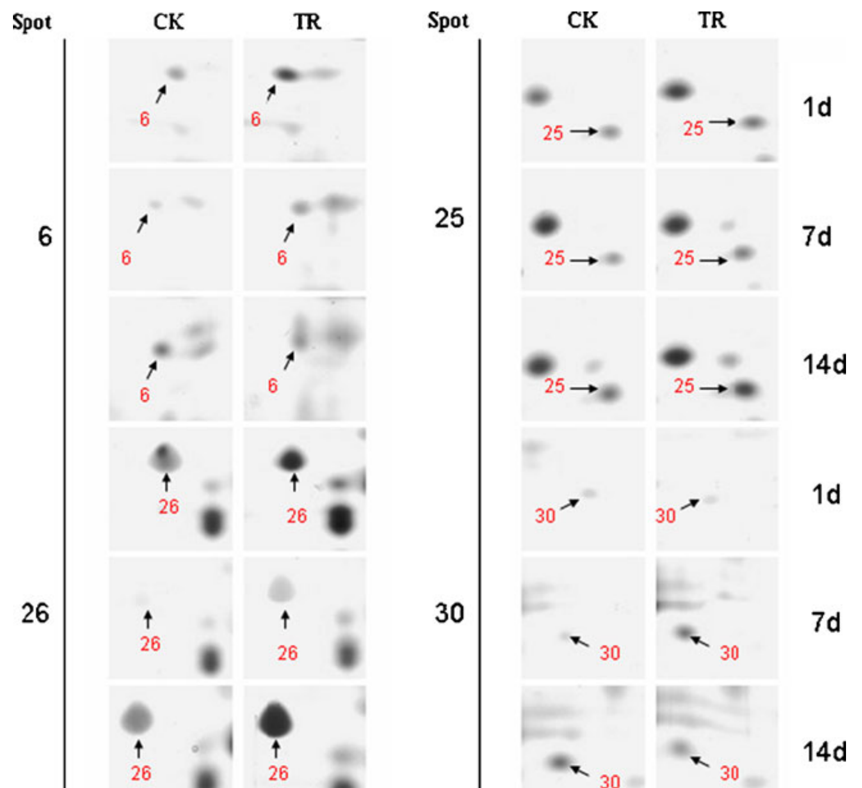

Fig. 3 Partial comparisons between images of some differentially expressed proteins of Dular leaves in CK and TR groups at three treatment stages

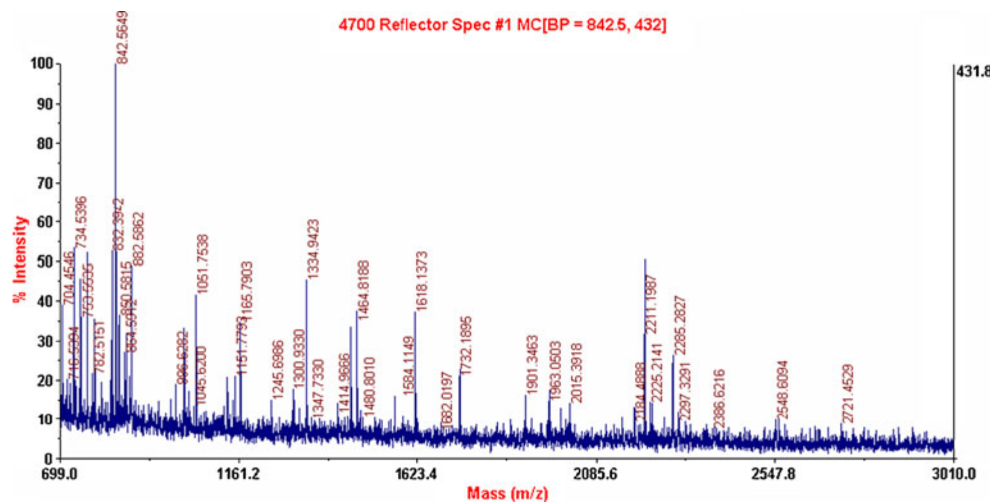

Fig. 4 Identification of putative ribose-5-phosphate isomerase by MALDI-TOF/MS

which catalyzes the reversible hydration of carbon dioxide: $CO_2 + H_2O \longleftrightarrow HCO_3^- + H^+$, was downregulated in Lemont after 7- and 14-day UV-B treatment, but it did not affect the photosynthesis (Cheng 2000).

Plastid-specific 30S ribosomal proteinII (PSRP-2, spot No. 6), acting in the light-regulation of chloroplast protein synthesis, upregulated expression in Lemont at all three treatment stages, but insignificantly in Dular

Table 2 Function classification and expressing patterns of proteins in rice leaves in response to UV-B

Functions	Protein spots identification	Expressing patterns in Lemont CK → TR			Expressing patterns in Dular CK → TR		
		1d	7d	14d	1d	7d	14d
Photosynthesis	16 Carbonic anhydrase	–	↓	↓	↑	–	–
	29 Carbonic anhydrase	–	–	–	↑	↓	–
	6 Plastid-specific 30S ribosomal protein 2	↑	↑	↑	↑	↑	
	20 Oxygen-evolving complex protein 1	–	–	↑	–	–	–
	28 RuBisCO subunit binding-protein alpha subunit	↑	↑	↑	–	↑	–
	30 Rubisco large subunit	–	–	↑	–	↑	–
	17 Putative Peptidyl-prolyl cis-trans isomerase	↑	↑	↑	–	–	–
Glycometabolism	10 Putative ribose-5-phosphate isomerase	↑	↑	↑	–	–	–
	15 Putative inorganic pyrophosphatase	↑	↑	↑	–	–	–
	24 Putative phosphoglycerate mutase	–	–	↑	–	↓	↓
	31 Dihydrolipoyl dehydrogenase	↓	↓	↓	↑	–	–
Protein metabolism	2 Aminotransferase y4Ub	–	–	↑	–	–	–
	23L-threonine 3-dehydrogenase (Quinone oxidoreductase)	–	↑	↑	–	↑	↑
Lipid metabolism	4 Putative delta 12 oleic acid desaturase	–	–	↑	–	–	–
Signal transduction and regulation	5 Vacuolar ATPase B subunit	↑	↑	↑	–	–	–
	14 Putative tyrosine phosphatase	–	↑	↑	↑	↓	–
	26 Glycine-rich protein	↓	↑	↑	–	↑	↑
Cell defense	9 Putative 4-coumarate coenzyme A ligase	↑	↑	↑	↑	↑	–
	13 Glutamate dehydrogenase	↓	↓	–	–	–	–
	21 Glutamine synthetase	–	↑	↑	–	–	–
	25 Peroxiredoxin-2C	–	↑	↑	–	↑	↑
	27 GSH-dependent dehydroascorbate reductase 1	↓	–	↑	↑	–	↓
	37 Superoxide dismutase [Mn]	↑	↑	↑	–	–	↑
Development	11 Putative fruit protein PKIWI502	↑	↑	↑	↑	↑	↓

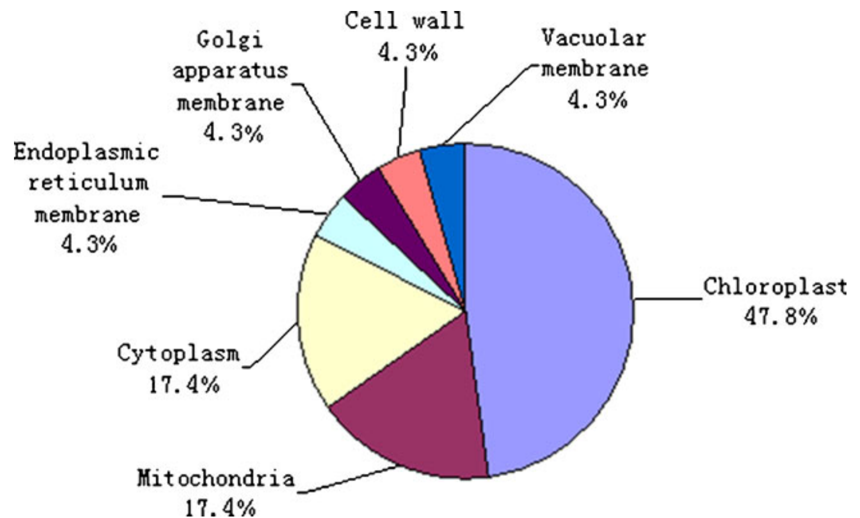

Fig. 5 Subcellular localizations of the differentially expressed proteins

after 14-day treatment. This might indicate that the chloroplast protein synthesis in Lemont was induced by the enhanced UV-B radiation, and that Lemont had a stronger resistance to UV-B than Dular did. Peptidyl-prolyl cis-trans isomerase (spot No. 17) is a type of chloroplast membrane protein that controls protein folding and plays a role in transporting proteins to chloroplast. Its abundance directly affects chloroplast membrane. This enzyme was expressed upregulatedly in Lemont only. The results showed that, in addition to chloroplast protein synthesis, under UV-B radiation stress, the biological function of proteins in Lemont was maintained by regulating the protein folding.

As far as glycometabolism is concerned, ribose-5-phosphate isomerase (spot No. 10) and inorganic pyrophosphatase (spot No. 15) were increased in Lemont only. The spot No. 10 enzyme involves with the interconversion of ribose-5-phosphate and ribulose-5-phosphate in Calvin cycle and pentose phosphate pathway. And ribose-5-phosphate is used in the synthesis of nucleotide and nucleic acid. The biosynthetic pathway becomes more active when tissue injury, repair, and regeneration occurs, which turns the carbohydrate metabolism to favor a more effective DNA repair. 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 UV-B radiation (Aguiler et al. 1999).

In cell defense, the 4-coumarate coenzyme A ligase (4CL, spot No. 9), plays a key role in channeling the carbon flow into diverse branch pathways of phenylpropanoid metabolism. It serves the important functions in the biosynthesis of plant phenypropanoids, such as lignin for structural support and flavonoid for UV protection. 4CL maintained a high expression in Lemont during all treatment stages, while no changes were found in Dular after 14-day UV-B treatment. It suggested that UV-B-tolerant rice, Lemont, was able to reduce UV-B radiation flux by accumulating phenolic acids and flavonoids resulting from the continuous high expression of 4CL (Rodrigo 1994), and that the UV-B-sensitive rice, Dular, displayed a weak tolerance to UV-B radiation due to the nonimprovement of 4CL expression. In the flavonoid biosynthesis in phenylpropanoid metabolic pathway, some ammonia is produced by nonoxidative deamination of certain amino acids. Glutamine synthetase (spot No. 21), a key enzyme for ammonium assimilation in higher plants, can regulate ammonium concentration in rice leaves. The content of this enzyme increased in Lemont after 7- and 14-day UV-B treatment, which might impel the nitrogen metabolism propitious to flavonoid synthesis. The expression of glutamate dehydrogenase (spot No. 13) was downregulated, which released ammonium by catalyzing glutamate oxidation. The two spots were not changed in Dular, which further indicated that phenylpropanoid metabolism was more active in Lemont than in Dular.

In addition, the proteins that scavenge ROS, including peroxiredoxin-2C (Prxc, spot No. 25), GSH-dependent dehydroascorbate reductase 1 (IDR, spot No. 27), and superoxide dismutase (SOD, spot No. 37), are effective in repairing tissue injury by ROS. Prxc and SOD increased in both rice accessions, while IDR decreased in Dular after 14-day UV-B treatment. The difference might again contribute to the stronger UV-B resistance in Lemont than Dular.

In regard to lipid metabolism, delta 12 oleic acid desaturase (spot No. 4), which involves in the desaturation of oleic acid to linoleic acid (Rock et al. 1996), was found only to increase in Lemont after 14-day UV-B treatment. As a result, the production of unsaturated fatty acid in Lemont

might be raised, contributing to the maintenance of fluidity and stability of membranes resulting in a reduced vulnerability to UV-B radiation.

For protein metabolism, L-threonine 3-dehydrogenase (Quinone oxidoreductase, spot No. 23) and aminotransferase y4uB (spot No. 2) are related to amino acid degradation. The former, which increased in both rice accessions, can cause injury to plants by stimulating ROS production as well as hydrogen peroxide (H_2O_2) formation during L-threonine degradation. The spot No. 2 enzyme, which increased in Lemont, catalyzes the reaction between amino acid and α-keto acid. The abundance of the two enzymes might contribute to the protein degradation in the rice under UV-B stress.

On signal transduction, tyrosine phosphatase (spot No. 17) is an important negative regulator of mitogen-activated protein kinases (MAPK) (Tena et al. 2001; Huang et al. 2000) and a critical signaling component in ABA-induced stomatal closure. MAPK cascades play an important role in mediating stress responses in eukaryotic organisms. MAPK is believed to mediate ABA-induced H_2O_2 generation in guard cells, and in turn, the production of H_2O_2 activates MAPK, thus forming a positive feedback loop (Zhang et al. 2009). The constitutive activation of MAPK cascades can cause damage to the cell. Tyrosine phosphatase acts as an inhibitor of H_2O_2 production by playing a role in MAPK dephosphorylation (Shi et al. 2004). This enzyme increased in Lemont during the treatment stages, but decreased in Dular after 7-day UV-B treatment. This might infer that UV-B-sensitive rice was vulnerable to UV-B damage due to its deficiency in ROS scavenging mechanism.

The vacuolar ATPase B subunit (spot No. 5) involves in Ca(2^+) channel, NO, and ABA signaling pathways (Zhao et al. 2006). Therefore, rice resistance to UV-B stress might also relate to these pathways (Mackerness et al. 2001). Glycine-rich protein (spot No. 26) increased in the two rice accessions exposed to UV-B, contributing to the extracellular signaling transduction, damage repair, RNA shearing, ripening, and gene expression (Ringli et al. 2001; Park et al. 2001).

In conclusion, pathway of protein expression under UV-B stress seems to differ in different rice cultivars. The enhancement or high intensity of the proteins that related to protein metabolism, fatty acid metabolism, photosynthesis, cell defense, and signal transduction might contribute to the rice's tolerance to the increased UV-B exposure. However, the reverse was true in UV-B-sensitive rice (Fig. 6). Besides, there are some other proteins affected by UV-B stress both in rice accessions that also involved in the physiological resistance to UV damage, indicating that a common cell defense mechanism exists in different rice cultivars. To validate the specific proteins and confirm the functions in enhancing rice UV resistance, further research applying the western blot analysis and/or enzyme-linked immunosorbent assay (ELISA) is in order. It would, then, help to develop an effective molecular method in breeding rice accessions with stable yield when they were exposed to excessive solar UV-B exposure.

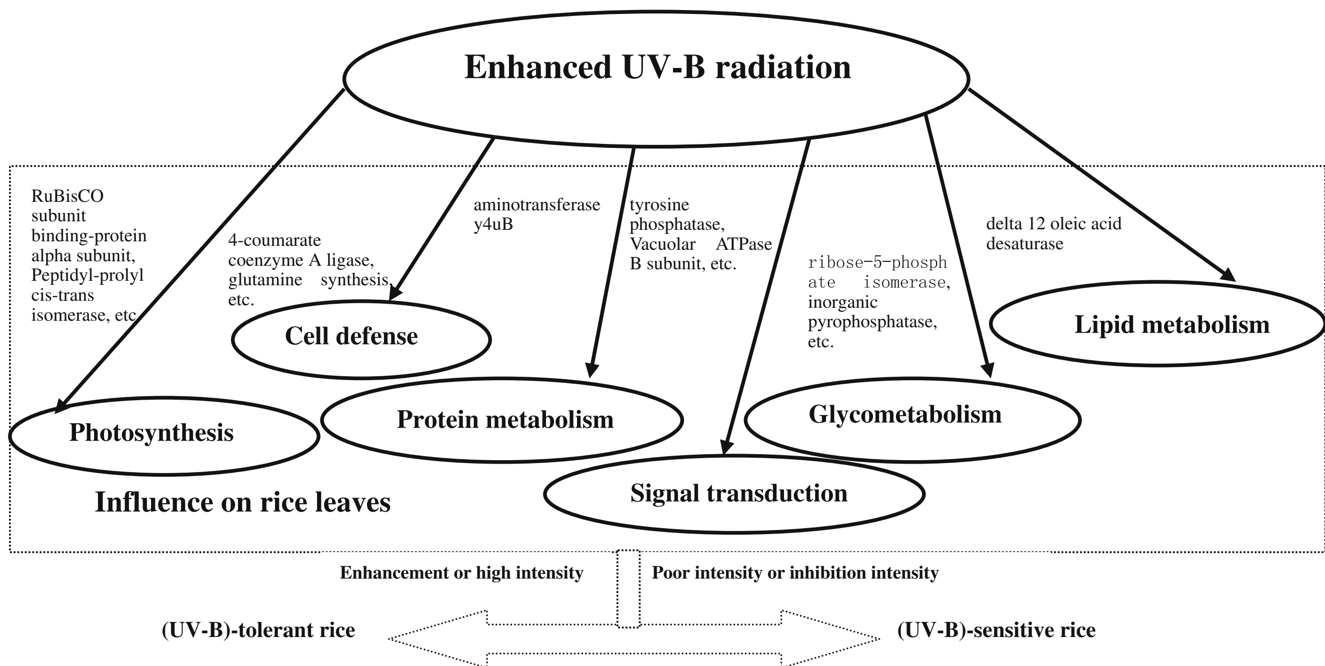

Fig. 6 The pathway of UV-induced protein expression in UV-B-tolerant and UV-B-sensitive rice cultivars

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References

Agrawal SB, Rathore D (2007) Changes in oxidative stress defense system in wheat (*Triticum aestivum* L.) and mung bean (*Vigna radiata* L.) cultivars grown with and without mineral nutrients and irradiated by supplemental ultraviolet-B. Environ Exp Bot 59:21–33

Aguiler J, Jiménez C, Figueroa FL, Lebert M, Häder D-P (1999) Effect of ultraviolet radiation on thallus absorption and photosynthetic pigments in the red alga *Porphyra umbilicalis*. J Photochem Photobiol B Biol 48:75–82

Barnes PW, Maggard S, Holma SR, Vergara B (1993) Intraspecific variation in sensitivity to UV-B radiation in rice. Crop Sci 33:1041–1046

Bassmam JH, Edward GE, Robberecht R (2002) Long term exposure to enhanced UV-B radiation is not detrimental to growth and photosynthesis in Douglas-fir. New Phytol 154:107–120

Bradford MM (1976) A rapid and sensitive method for the quantisation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Casati P, Zhang X, Burlingame AL, Walbot V (2005) Analysis of leaf proteome after UV-B irradiation in maize lines differing in sensitivity. Mol Cell Proteomics 4:1673–1685

Cassi-Lit M, Whitecross MJ, Nayudu M, Tanner GJ (1997) UV-B irradiation induces differential leaf damage, ultrastructural changes and accumulation of specific phenolic compounds in rice cultivars. Aust J Plant Physiol 24:261–274

Cheng XW (2000) Effects of environmental factors on carbonic anhydrase activities in chloroplast of rice leaves. J Hubei Nor Univer (Nat Sci) 20:40–42, in Chinese with English abstract

Correia CM, Torres-Pereira MS, Torres-Pereira JMG (1999) Growth, photosynthesis and UV-B absorbing compounds of Portuguese Barbela wheat exposed to ultraviolet-B radiation. Environ Pollut 104:383–388

Dai Q, Coronel VP, Vergara BS, Barnes PW, Quintos AT (1992) Ultraviolet-B radiation effects growth and physiology of four rice cultivars. Crop Sci 32:1269–1274

Fang CX, Wu XC, Zhang HL, Xiong J, Wu WX, Lin WX (2009) UV-induced differential gene expression in rice cultivars analyzed by SSH. Plant Growth Regul 59:245–253

Fedina IS, Grigorova ID, Georgieva KM (2003) Response of barley seedlings to UV-B radiation as affected by NaCl. J Plant Physiol 160:205–208

Feng YW, Komatsu S, Furukawa T, Koshiba T, Kohno Y (2008) Proteome analysis of proteins responsive to ambient and elevated ozone in rice seedlings. Agr Ecosyst Environ 125:255–265

Frohnmeyer H, Staiger D (2003) Ultraviolet-B radiation-mediated responses in plants. Balancing damage and protection. Plant Physiol 133:1420–1428

Ganguly AR, Steinhaeuser K, Erickson DJ III, Branstetter M, Parish ES, Singh N, Drake JB, Buja L (2009) Higher trends but larger uncertainty and geographic variability in 21st century temperature and heat waves. Proc Nat Acad Sci USA 106:15555–15559

He J, Huang LK, Chow WS, Whitecross MI, Anderson JM (1994) Responses of rice and pea plants to hardening with low doses of ultraviolet-B radiation. Aust J Plant Physiol 21:563–574

Huang Y, Li H, Gupta R, Morris PC, Luan S, Kieber JJ (2000) ATMPK4, an Arabidopsis homolog of mitogen-activated protein kinase, is activated in vitro by AtMEK1 through threonine phosphorylation. Plant Physiol 122:1301–1310

Jansen MAK, Gaba V, Greenberg BM (1998) Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends Plant Sci 3:131–135

Kaspar S, Matros A, Mock H-P (2010) Proteome and flavonoid analysis reveals distinct responses of epidermal tissue and whole leaves upon UV-B radiation of barley (*Hordeum vulgare* L.) seedlings. J Proteome Res 9:2402–2411

Ke YQ, Han GQ, He HQ, Li JX (2009) Differential regulation of proteins and phosphoproteins in rice under drought stress. Biochem Biophys Res Commun 379:133–138

Khan MMK, Komatsu S (2004) Rice proteomics: recent developments and analysis of nuclear proteins. Phytochemistry 65:1671–1681

Kornberg A (1962) On the metabolic significance of phosphorolytic and pyrophosphorolytic reactions. In: Kasha M, Pullman E (eds) Horizons in biochemistry. Academic, New York, pp 251–287

Kumari R, Singh S, Agrawal SB (2009) Effects of supplemental ultraviolet-B radiation on growth and physiology of *Acorus calamus* L. (sweet flag). Acta Biologica Cracoviensia Ser Bot 51(2):19–27

Lee DG, Ahsan N, Lee SH, Lee JJ, Bahk JD, Kang KY, Lee BH (2009) Chilling stress-induced proteomic changes in rice roots. J Plant Physiol 166:1–11

Lee K, Bae DW, Kim SH, Han HJ, Liu X, Park HC, Lim CO, Lee SY, Chung WS (2010) Comparative proteomic analysis of the short-term responses of rice roots and leaves to cadmium. J Plant Physiol 167:161–168

Li Y, Yue M, Wang XL (1998) Effects of enhanced ultraviolet-B radiation on crop structure, growth and yield components of spring wheat under field conditions. Field Crops Res 57:253–263

Li Y, Zu YQ, Chen HY, Chen JJ, Yang JL, Hu ZD (2000) Intraspecific responses in crop growth and yield of 20 wheat cultivars to enhanced ultraviolet-B radiation under field conditions. Field Crops Res 67:25–33

Liang CJ, Huang XH, Zhou Q (2006) Effect of cerium on photosynthetic characteristics of soybean seedling exposed to supplementary ultraviolet-B radiation. J Environ Sci 18:1147–1151

Lin WX, Liang YY, Kim KU (1999) Genetics of resistance of *Oryza sativa* to increased UV-B radiation and its physiobiochemical characteristics. Chin J Appl Ecol 10:31–34, in Chinese with English abstract

Mackerness SAH, Jordan BR, Thomas B (1999) Reactive oxygen species in the regulation of photosynthetic genes by ultraviolet-B radiation (UV-B: 280–320 nm) in green and etiolated buds of pea (*Pisum sativum* L.). J Photochem Photobiol B 48(2–3):180–188

Mackerness SAH, John CF, Jordan B, Thomas B (2001) Early signaling components in ultraviolet-B responses: distinct roles for different reactive oxygen species and nitric oxide. FEBS Lett 489:237–242

Markham KR, Tanner GJ, Caasi-Lit M, Whitecross MI, Nayudu M, Mitchell KA (1998) Possible protective role for 3′,4′-dihydroxyflavones induced by enhanced UV-B in a UV-tolerant rice cultivar. Phytochemistry 49:1913–1919

Masakazu G, Hiroshi E, Shyuichi K, Keiji T, Natsumi O, Yutaka Y, Satoru OG, Yahaya MS, Osamu M (2001) Protective effect of silicon on phenolic biosynthesis and ultraviolet spectral stress in rice crop. Plant Sci 164:349–356

Olszyk DM, Ingram KT (1991) Effects of UV-B and global change on rice production: the EPA/IRRI Cooperative Research Plan. In: Ilyas M (ed.) Ozone depletion implications of the tropics. University Science Malaysia and U.N. Environmental Programme: 234–253

Park AR, Cho SK, Yun UJ, Jin MY, Lee SH, Sachetto-Martins G, Park OK (2001) Interaction of the Arabidopsis receptor protein kinase Wak1 with a glycine rich protein, AtGRP3. J Biol Chem 276:26688–26693

Peng Q, Zhou Q (2009) Influence of lanthanum on chloroplast ultrastructure of soybean leaves under ultraviolet-B stress. J Rare Earths 27:304–307

Pontin MA, Piccoli PN, Francisco R, Bottini R, Martinez-Zapater JM, Lijavetzky D (2010) Transcriptome changes in grapevine (*Vitis vinifera* L.) cv. Malbec leaves induced by ultraviolet-B radiation. BMC Plant Biol 10:224

Qu Y, Feng H, Wang Y, Zhang M, Cheng J, Wang X, An L (2006) Nitric oxide functions as a signal in ultraviolet-B induced inhibition of pea stems elongation. Plant Sci 170:994–1000

Ringli C, Keller B, Ryser U (2001) Glycine-rich proteins as structural components of plant cell walls. Cell Mol Life Sci 58:1430–1441

Rock CO, Tsay JT, Heath R, Jackowski S (1996) Increased unsaturated fatty acid production associated with a suppressor of the fabA6 (Ts) mutation in *Escherichia coli*. J Bacteriol 178:5382–5387

Rodrigo L (1994) Accumulation of UV-absorbing flavonoids induced by UV-B radiation. Planta 194:498–503

Salekdeh GH, Siopongco J, Wade LJ, Ghareyazie B, Bennett J (2002) A proteomic approach to analyzing drought- and salt-responsiveness in rice. Field Crops Res 76:199–219

Sato T, Kang HS, Kamagai T (1994) Genetic study of resistance to inhibitory effects UV radiation in rice (*Oryza sativa* L.). Physiol Plant 91:234–238

Sato T, Ueda T, Fukuta Y, Kumagai T, Yano M (2003) Mapping of quantitative trait loci associated with ultraviolet-B resistance in rice (*Oryza sativa* L.). Theor Appl Genet 107:1003–1008

Schmitz-Hoerner R, Weissenböck G (2003) Contribution of phenolic compounds to the UV-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated UV-B levels. Phytochemistry 64:243–255

Scotto J, Cotton G, Urbach F, Berger D, Fears T (1988) Biological effective ultraviolet radiation: surface measurements in the United States, 1974 to 1985. Science 239:762–764

Shi W, Jia W, Liu X, Zhang S (2004) Protein tyrosine phosphatases involved in signaling of the ABA-induced H_2O_2 generation in guard cells of *Vicia faba L*. Chin Sci Bull 147:1841–1846

Singh A, Sarkar A, Singh S, Agrawal SB (2010) Investigation of supplemental ultraviolet-B-induced changes in antioxidative defense system and leaf proteome in radish (*Raphanus sativus* L. cv Truthful): an insight to plant response under high oxidative stress. Protoplasma 245:75–83

Tena G, Asai T, Chiu WL, Sheen J (2001) Plant mitogen-activated protein kinase signaling cascades. Curr Opin Plant Biol 4:392–400

Teramura AH, Ziska LH, Sztein AE (1991) Changes in growth and photosynthetic capacity of rice with increased UV-B radiation. Physiol Plant 83:373–380

Vorum H, Hager H, Christensen BM, Nielsen S, Honoré B (1999) Human calumenin localizes to the secretory pathway and is secreted to the medium. Exp Cell Res 248:473–481

Wu XC, Lin WX, Huang ZL (2007) Influence of enhanced ultraviolet-B radiation on photosynthetic physiologies and ultrastructure of leaves in two different resistively rice cultivars. Acta Ecol Sin 27:0554–0564, in Chinese with English abstract

Xu K, Qiu BS (2007) Responses of superhigh-yield hybrid rice liangyoupeijiu to enhancement of ultraviolet-B radiation. Plant Sci 172:139–149

Xu C, Sullivan JH, Garrett WM, Caperna TJ, Natarajan S (2008) Impact of solar ultraviolet-B on the proteome in soybean lines differing in flavonoid contents. Phytochemistry 69:38–48

Yu CL, Yan SP, Wang CC, Hu HT, Sun WN, Yan CQ, Chen JP, Yang L (2008) Pathogenesis-related proteins in somatic hybrid rice induced by bacterial blight. Phytochemistry 69:1989–1996

Zhang A, Jiang M, Zhang J, Tan M, Hu X (2009) Mitogen-activated protein kinase is involved in abscisic acid-induced antioxidant defense and acts downstream of reactive oxygen species production in leaves of maize plants. Curr Opin Plant Biol 12:421–426

Zhao Y, Han B, Li SF, Wang YF, He JF (2006) The role in the response of vacuolar H^+-ATPase to the abiotic stress and the signal transduction in plants. Plant Physiol Commun 42:812–816, in Chinese with English abstract

Zivy MD, Vienne D (2000) Proteomics: a link between genomics, genetics and physiology. Plant Mol Biol 44:575–580