UV Radiation-Responsive Proteins in Rice Leaves: 
A Proteomic Analysis

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Depletion of stratospheric ozone has led to increased UV radiation reaching the surface of the Earth. This may damage plants. Using physiological, proteomic and quantitative real-time PCR (qPCR) methods, we systematically studied the response of 16-day-old rice seedlings to UV [0.67 W m⁻² biologically effective UVB (UVBₑ) and 0.28 W m⁻² UVA] exposure for 6, 12 and 24 h. UV exposure resulted in the appearance of light brown patches on leaves, a decrease in the net photosynthetic rate (Pn), lipid peroxidation, accumulation of UV-absorbing compounds (including flavonoids and other phenolic pigments) and differential expression of 22 proteins. Both physiological and molecular responses became stronger with increasing UV exposure time, indicating the effects of UV accumulation on plants. UV-induced responses included (i) phytohormone-regulative responses (up-regulation of proteins related to phytohormone synthesis such as IAA and ethylene); (ii) injurious responses (photosynthesis suppression, lipid peroxidation and visible injury); and (iii) protective responses (accumulation of UV-absorbing compounds and differential expression of proteins involved in detoxification/antioxidation, defense, protein processing, RNA processing, carbohydrate metabolism and secondary metabolism). The identification of UV-responsive proteins provided a better understanding of the molecular mechanism of plant responses to UV stress. Proteomic and qPCR analysis identified one up-regulated and two induced proteins with important functions: tryptophan synthase chain a, glyoxalase I (detoxification/antioxidation) and a Bet v I family protein (defense). These results will contribute to future research into their roles in UV stress responses in plants.

Keywords: Glyoxalase I • Oryza sativa • Proteome • Stress • UV radiation.

Abbreviations: Cond, stomatal conductance; 2-DGE, two-dimensional gel electrophoresis; DTT, dithiothreitol; Hsp, heat shock protein; MALDI-TOF, matrix-assisted laser desorption ionization time of flight; MDA, malondialdehyde; PDF, pigment-dispersing factor; 6-PGDH, 6-phosphogluconate dehydrogenase; Pn, net photosynthetic rate; PR protein, pathogen-related protein; qPCR, quantitative real-time PCR; ROS, radical oxygen species; Rubisco LSU, ribulose-1, 5-bisphosphate carboxylase large subunit; SAMS, S-adenosylmethionine synthetase; TCA, trichloroacetic acid; TFA, trifluoroacetic acid; UVBₑ, biologically effective UVB.

Introduction

Depletion of stratospheric ozone has led to a significant increase in UV radiation reaching the surface of the Earth (Madronich et al. 1998, McKenzie et al. 1999). This is predicted to continue in the future (Caldwell et al. 2003, McKenzie et al. 2003). Elevated UV is potentially deleterious to many organisms and has received considerable attention in the past decade. UV augmentation can restrain growth of plants, including some important crops such as rice and maize, resulting in severe reduction in yield (Ambasht and Agrawal 1997, Kumagai et al. 2001, Gao et al. 2004, Hidema et al. 2007). On the other hand, plants have developed protective mechanisms against UV stress, such as enhancement of the antioxidant system (Brosché and Strid 2003) and accumulation of UV-absorbing compounds (Frohnmeyer and Staiger 2003). Both injurious and protective responses are usually the consequences of UV-induced alteration of gene expression (A.-H.-Mackerness 2000, Kalbina and Strid 2006). Therefore, research on gene expression is essential for better comprehension of the mechanism of the response of plants to UV stress, which is beneficial for developing future strategies of crop improvement.

UV radiation may induce plants to generate signal transduction intermediates such as nitric oxide, reactive oxygen species (ROS) and ethylene, followed by down-regulation of photosynthesis-related proteins such as light-harvesting Chl a/b-binding protein and up-regulation of protective proteins such as pathogen-related protein-1 (PR-1) and pigment-dispersing factor 1.2 (PDF 1.2) at the mRNA level.
(Desimone et al. 1998, Surplus et al. 1998, A.-H.-Mackerness et al. 1999, Frohnmeyer et al. 1999, A.-H.-Mackerness 2000, A.-H.-Mackerness et al. 2001, Kalbina and Strid 2006). UV radiation may also enhance expression of transcripts of some senescence-associated genes and result in a cellular decline in Arabidopsis (John et al. 2001). In addition, recent transcriptional analyses with plants demonstrate that UV radiation affects expression of a large number of genes involved in a wide range of cellular processes, such as DNA damage and binding, photosynthesis, stress and cellular detoxification (Casati and Walbot 2004, Casati et al. 2006, Kilian et al. 2007). These analyses at the mRNA level have contributed to our knowledge of the expression pattern of UV-regulated genes. However, in response to environmental factors, the level of protein production does not always agree well with the level of mRNA expression (Yan et al. 2006, Lee et al. 2009). Proteins are not only the products of genes, but also the key players in metabolism. Proteomic analysis of total cellular proteins will help us to discover the expression patterns of UV-induced genes at the protein level. Recently, the effects of early UV stress (with 8 h) on maize seedlings have been investigated by a proteomic method, and 60 differentially expressed proteins were identified (Casati et al. 2005). Rice is not only the most important food crop in the world, but also a model monocot plant for molecular studies, which is different from maize in terms of genomic structures and growth habitats. Furthermore, most of our main foods are provided by monocot crops, but data from the effects of UV stress on the model monocot plant rice are limited at both the mRNA and protein levels. Therefore, a UV-induced proteomic study on rice in conjunction with a quantitative real-time PCR (qPCR) method will provide new insight into the inner mechanism underlying injurious and protective responses of crops during UV stress.

We conducted a systematic study on early (6 and 12 h) and late (24 h) UV stress responses in rice plants grown under controlled experimental conditions via physiological, proteomic and qPCR methods. Our objectives are: to determine whether UV-induced morphological, physiological and molecular (mRNA and protein) responses are time dependent; to identify UV-induced differential proteins at different stages; and to detect correlations between mRNA and proteins during UV stress.

**Results**

**Morphological and physiological responses of rice leaves during UV exposure**

UV-induced morphological and physiological responses are time dependent. All plants looked healthy at 6 and 12 h UV treatment, but light brown patches (mainly around the central vein) were observed on the leaves in treated plants at 24 h, not accompanied by chlorotic symptoms (Fig. 1). To evaluate the intrinsic adverse effect of UV radiation on plants, we measured the leaf net photosynthesis rate ($P_n$), stomatal conductance ($\text{Cond}$), the maximum photochemical efficiency of PSII ($F_v/F_m$), Chl content and lipid peroxidation production [level of malondialdehyde (MDA)]. $P_n$ and $F_v/F_m$ significantly decreased ($P < 0.001$) at 12 and 24 h (Fig. 2A, C), following by a significant decline of $\text{Cond}$ during UV stress (Fig. 2B), which suggested that the UV-induced functional change in the stomata occurred earlier than changes in PSII and $P_n$. The Chl content showed no significant decrease during UV stress, which was consistent with no chlorotic symptoms on leaves (Fig. 2D). MDA significantly increased by 69.8% (7.79 ± 0.59 vs. 13.23 ± 1.05; $P < 0.01$) at 24 h (Fig. 2E), accompanied by the occurrence of light brown patches on leaves, suggesting the initiation of oxidative damage. We also measured the content of protective UV-absorbing compounds, which significantly accumulated at early and late stages (12 and 24 h) (Fig. 2F).

**Proteomic responses of rice leaves during UV exposure**

To study the molecular mechanisms underlying the morphological and physiological UV-induced responses of rice leaves, we used two-dimensional electrophoresis (2-DGE)
to compare all extractable rice leaf proteins from control and UV-treated plants at 6, 12 and 24 h of UV exposure. We found that 20 proteins were up-regulated (P < 0.05, and 1.5- to 4.9-fold), and three proteins were induced during UV exposure (Fig. 3). We also detected three significantly decreased proteins, whose intensity decreased by nearly 1.5 fold. (P < 0.05, and /C0 1.3- to /C0 1.5-fold) (Supplementary Fig. S1). Proteomic changes in rice leaves were related to time of UV exposure. Two spots (spots 1 and 2) were up-regulated and one spot (spot S1) was significantly decreased at 6 h; two spots (spots 3 and 4) were up-regulated, two spots (spots 5 and 6) were induced and one spot (spot S2) was significantly decreased at 12 h. At 24 h, spots 3 and 4 continued to be up-regulated, and the abundance of spots 5 and 6 increased; in addition, another 16 spots (spots 7–22) were up-regulated, one spot (spot 23) was induced and one spot (spot S3) was significantly decreased (Fig. 3 and Supplementary Fig. S1). The change in UV-responsive proteins was consistent with the morphological and physiologal response of rice leaves to UV exposure, which indicated effects of UV accumulation on rice plants.

Among up-regulated and significantly decreased protein spots, 23 spots were identified as 22 unique proteins by MALDI-TOF (matrix-assisted laser desorption ionization time of flight) mass spectrometry analysis (Table 1 and Supplementary Table S1). Spots 18 and 19 were endosperm luminal binding protein, which might have two isoforms. Some of these identified proteins were newly linked to UV exposure, such as tryptophan synthase α chain, S-adenosylmethionine synthetase (SAMS), chitinase, Bet v I allergen family protein, chaperonin 60 α subunit, heat shock protein ST1 (HspST1), endosperm luminal binding protein, 6-phosphogluconate dehydrogenase (6-PGDH), malate dehydrogenase, aconitate hydratase, phosphoglycerate dehydrogenase, isoprenoid biosynthesis-like protein and manganese-stabilizing protein/PSII polypeptide (Table 1 and Supplementary Table S1). This finding is beneficial for a better comprehension of the molecular mechanisms of plants against UV stress. The identified UV-induced differential proteins were categorized into eight functional classes: phytohormone synthesis, photosynthesis, detoxification/antioxidation, defense, protein processing,
RNA processing, carbohydrate metabolism and secondary metabolism (Table 1). These proteins with various functions were involved in UV-induced phytohormone-regulative, injurious and protective responses.

The phytohormone-regulative response showed that tryptophan synthase α chain increased by 2.3- and 4.0-fold, and SAMS increased by 1.8- and 2.2-fold at 12 and 24 h, respectively. Tryptophan synthase α chain (apot 3) and SAMS (spot 4) were involved in phytohormone synthesis. The injurious response showed an increase in abundance of one 37 kDa ribulose-1, 5-bisphosphate carboxylase large subunit (Rubisco LSU) fragment (spot 2) and a decrease of Rubisco activase, putative chloroplast precursor (spot S1) and manganese-stabilizing protein/PSII polypeptide (spot S2) at 6 or 12 h, which were related to photosynthesis. The protective responses showed the differential expression of proteins involved in detoxification/antioxidation, defense, protein processing, RNA processing, carbohydrate metabolism and secondary metabolism.

Glyoxalase I (spot 5), which participates in detoxification/antioxidation, was induced at 12 h, and increased in abundance at 24 h. Defense proteins, including chitinase (spot 6) and Bet v 1 allergen family protein (spot 23), were induced at 12 or 24 h. Proteins associated with protein processing were the most abundant functional category, which included chloroplast stromal Hsp70-related protein (spot 7), Hsp70 mitochondrial precursor (spot 12), putative Hsp70 mitochondrial precursor

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**Fig. 3** 2-DGE maps of rice leaf proteins during UV exposure. Quantitative image analysis revealed 23 differential spots with >1.5-fold changes and $P < 0.05$ (Student’s t-test). Numbered spots are identified in Table 1.
Carbohydrate metabolism were all up-regulated at 24 h. Exposure. Reprogramming of carbohydrate metabolism might be involved in adaptation of rice plants to UV stress. Among the identified proteins, tryptophan synthase α chain (spot 3), glyoxalase I (spot 5) and Bet v 1 allergen family protein (spot 23) were selected for further investigation of their UV-responsive patterns at the mRNA level. The three proteins (spot 3), glyoxalase I and Bet v 1 allergen family protein (spot 5) significantly increased at 12 and 24 h, but their change in expression indirectly reflected the alteration in carbohydrate metabolism in response to UV exposure. Reprogramming of carbohydrate metabolism might be involved in adaptation of rice plants to UV stress.

### Gene expression of three UV-responsive proteins at the mRNA level

Among the identified proteins, tryptophan synthase α chain (spot 3), glyoxalase I (spot 5) and Bet v 1 allergen family protein (spot 23) were selected for further investigation of their UV-responsive patterns at the mRNA level. The three proteins were considered to play an important role in response to UV stress in rice plants. At the mRNA level, tryptophan synthase α chain and glyoxalase I significantly increased at 12 and 24 h, but

### Table 1: Identification of UV-induced differentially expressed proteins in rice leaves

<table>
<thead>
<tr>
<th>Functional category</th>
<th>Spot No.</th>
<th>Folda</th>
<th>Accession No.</th>
<th>Matched protein description</th>
<th>Scoreb</th>
<th>SCc (%)</th>
<th>PIobs/cal</th>
<th>Mobs/cal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytohormone</td>
<td>3</td>
<td>1.03</td>
<td>2.28*</td>
<td>NP_001059049 Tryptophan synthase α chain</td>
<td>106</td>
<td>51</td>
<td>5.16/6.2</td>
<td>32/34.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>−1.05</td>
<td>1.76*</td>
<td>CAJ4588 SAMS</td>
<td>81</td>
<td>30</td>
<td>6.01/5.8</td>
<td>48/43.0</td>
</tr>
<tr>
<td>Photosynthesis</td>
<td>2</td>
<td>2.57*</td>
<td>−1.20</td>
<td>CAG34174 Rubisco LSU</td>
<td>93</td>
<td>38</td>
<td>6.72/6.2</td>
<td>37/53.3</td>
</tr>
<tr>
<td>Detoxification/antioxidation</td>
<td>5</td>
<td>−</td>
<td>Enhanced</td>
<td>NP_001061172 Glyoxalase I</td>
<td>74</td>
<td>51</td>
<td>5.86/5.5</td>
<td>38/32.9</td>
</tr>
<tr>
<td>Defense</td>
<td>6</td>
<td>−</td>
<td>Induced</td>
<td>AAB58238 Chitinase</td>
<td>73</td>
<td>41</td>
<td>5.62/7.7</td>
<td>28/28.0</td>
</tr>
<tr>
<td>Protein processing</td>
<td>9</td>
<td>1.21</td>
<td>1.16</td>
<td>NP_001066567 Chaperonin 60 α subunit</td>
<td>137</td>
<td>48</td>
<td>6.86/5.1</td>
<td>42/61.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.03</td>
<td>1.25</td>
<td>NP_001056601 Chaperonin 60 β subunit</td>
<td>131</td>
<td>47</td>
<td>5.02/5.6</td>
<td>66/64.3</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>−1.05</td>
<td>1.13</td>
<td>NP_001055140 Chloroplast stromal Hsp70-related protein</td>
<td>77</td>
<td>26</td>
<td>5.11/4.6</td>
<td>31/48.7</td>
</tr>
<tr>
<td>RNA processing</td>
<td>12</td>
<td>1.10</td>
<td>1.28</td>
<td>NP_001048274 Hsp70 mitochondrial precursor</td>
<td>168</td>
<td>47</td>
<td>5.67/5.5</td>
<td>71/72.9</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>14</td>
<td>1.34</td>
<td>1.43</td>
<td>AAO17017 Putative Hsp70 mitochondrial precursor</td>
<td>76</td>
<td>24</td>
<td>5.63/5.5</td>
<td>71/70.7</td>
</tr>
<tr>
<td>metabolism</td>
<td>16</td>
<td>1.00</td>
<td>1.02</td>
<td>NP_001047563 HspSTI</td>
<td>237</td>
<td>48</td>
<td>6.36/6.0</td>
<td>73/65.2</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>−1.22</td>
<td>1.47</td>
<td>AAB63469 Endosperm luminal binding protein</td>
<td>272</td>
<td>42</td>
<td>5.07/5.3</td>
<td>73/73.7</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>1.05</td>
<td>1.27</td>
<td>AAB63469 Endosperm luminal binding protein</td>
<td>272</td>
<td>42</td>
<td>5.12/5.3</td>
<td>73/73.7</td>
</tr>
<tr>
<td>Secondary</td>
<td>20</td>
<td>1.20</td>
<td>1.37</td>
<td>AAB66885 Glycine-rich protein</td>
<td>72</td>
<td>66</td>
<td>5.88/7.8</td>
<td>16/16.0</td>
</tr>
<tr>
<td>metabolism</td>
<td>21</td>
<td>1.56*</td>
<td>1.40</td>
<td>NP_001056586 Cytosolic 6-PGDH</td>
<td>154</td>
<td>63</td>
<td>6.16/5.9</td>
<td>53/53.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1.04</td>
<td>1.17</td>
<td>NP_001062517 Malate dehydrogenase</td>
<td>196</td>
<td>45</td>
<td>5.71/7.0</td>
<td>45/47.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.46</td>
<td>−1.19</td>
<td>NP_001048898 Cytoplasmic aconitate hydratase</td>
<td>90</td>
<td>27</td>
<td>6.16/6.5</td>
<td>103/106.9</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>1.27</td>
<td>1.23</td>
<td>BAD09434 Phosphoglycerate dehydrogenase</td>
<td>127</td>
<td>29</td>
<td>6.05/6.5</td>
<td>66/64.7</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>−1.07</td>
<td>−1.30</td>
<td>AAO72576 Isoprenoid biosynthesis-like protein</td>
<td>282</td>
<td>54</td>
<td>5.75/5.3</td>
<td>75/68.0</td>
</tr>
</tbody>
</table>

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[1] Consistent with the spot number in Fig. 3.
[2] Fold changes of the spot intensity between UV-treated leaves and the corresponding control at each time point.
[5] The protein score is −10 × log(P), where P is the probability that the observed match is a random event; protein scores >61 are significant (P < 0.05).
[7] Asterisk indicates spots with >1.5-fold change and P < 0.05 (Student’s t-test).
their fold change (20.0- and 77.2-fold, and 2.4- and 2.1-fold, respectively) were different from those at the protein level (2.3- and 4.0-fold; induced and increased); Bet v I allergen family protein was up-regulated by 9.7- and 170.4-fold at the mRNA level at 12 and 24 h UV exposure, and was induced at the protein level only at 24 h (Fig. 4). The subtle differences may account for differential turnover rates or RNA and protein stability (Bae et al. 2003). The up-regulated trend at the mRNA level of tryptophan synthase $\alpha$ chain, glyoxalase I and Bet v I allergen family protein was consistent with that at the protein level. The result confirmed the response of tryptophan synthase $\alpha$ chain, glyoxalase I and Bet v I allergen family protein to UV stress, which provided a good starting point to investigate further their roles in response to UV stress by genetic and other methods, potentially contributing to the development of future strategies for crop improvement.

**Discussion**

Proteomic analysis is an effective method to investigate the integral change in gene expression of plants during environmental stress at the protein level (Yan et al. 2006, Lee et al. 2009). Using a proteomic method, Casati et al. (2005) found some differential proteins of maize seedlings in early responses (within 8 h) to UV stress, and constructed the UV-responsive network at the protein level. The present study detected the enhanced expression of glyoxalase I at the mRNA and protein levels in response to UV stress at both early and late stages in rice plants, and also identified some new UV-responsive proteins including tryptophan synthase $\alpha$ chain, SAMS, chitinase, Bet v I allergen family protein and HspST1. These provided new insight into the protein and mRNA response of plants to UV exposure.

Rice seedlings in our study looked healthy and some proteins were differentially expressed at the early stage of UV exposure, which was consistent with the early response of maize seedlings to UV stress (Casati et al. 2005). The detailed difference in the change of proteins in the early response to UV might be due to the specific responses of rice seedlings and different intensity of UV stress. When UV exposure was prolonged to 24 h, morphological, physiological, proteomic and mRNA changes were all obviously enhanced in our experiment (Figs. 1–4), which indicated that UV-induced changes in rice seedlings were time dependent. These changes also reflected phytohormone-regulative, injurious and protective responses in rice seedlings during UV exposure.

**Fig. 4** Comparison of expression patterns of mRNA (A) and protein abundance (B) of UV-responsive proteins. Spot 3, tryptophan synthase $\alpha$ chain; spot 22, glyoxalase I; spot 23, Bet v I allergen family protein. Data are representative of three independent experiments and are shown as mean ± SE. * and ** represent $P < 0.05$ and $P < 0.01$ significance levels, respectively. Real-time PCRs were conducted using gene-specific primers (Table 2). Arrows indicate the protein spot on the 2-DGE map (Fig. 3).
Phytohormone-regulative response to UV stress

The phytohormone ethylene regulates plant responses to environmental stresses (Chen et al. 2005), and IAA controls many processes, including embryo development (Ljung et al. 2002). In our study, tryptophan synthase α chain (in relation to IAA synthesis and ROS creation) and SAMS (in relation to ethylene synthesis) presumably participated in phytohormone synthesis and signal transduction in rice seedlings during UV exposure, which might regulate UV-induced injurious and protective responses. SAMS catalyzes the formation of S-adenosylmethionine as the precursor for the phytohormone ethylene (Yan et al. 2006). Tryptophan synthase α chain catalyzes the last step of the synthesis of tryptophan as the precursor of IAA (Ljung et al. 2002). In addition, tryptophan is easily degraded by UVB, accompanied by ROS creation (Andley et al. 1984, Caldwell 1993). ROS, an important molecular signal in Arabidopsis during UV exposure, down-regulate harvesting Chl a/b-binding protein, and up-regulate PR-1 and PDF1.2 (A.-H.-Mackerness 2000). Up-regulation of PR-1 and PDF1.2 is also dependent on ethylene (A.-H.-Mackerness et al. 1999).

Injurious responses to UV stress

UV can depress photosynthesis in plants, and damage some cellular components, such as proteins and lipids (Andley et al. 1984, Ambasht and Agrawal 1997, Alexieva et al. 2001, Fedina et al. 2010). In this study, UV exposure led to inhibition of photosynthesis (a decrease in Pn, Cond and Fv/Fm; increase or decrease of photosynthesis-related proteins) of rice seedlings at the early stage; photosynthesis was more strongly suppressed, and oxidative damage (leaf injury and lipid peroxidation) occurred at the late stage. The decrease in Pn in our study might result from a UV-induced functional change of the stomata, PSI and photosynthesis-related proteins. The changes in photosynthesis-related proteins included a decrease in two proteins (spots S1 and S2) and an increase of the 37 kDa Rubisco LSU fragment. Ishida et al. (1999) also found that the LSU of Rubisco in the illuminated lysates of wheat chloroplasts is degraded into 37 and 16 kDa polypeptides by ROS. The 37 kDa LSU fragment detected in this study might be from the degradation of LSU induced by UV exposure through a similar pathway. The relatively high accumulation level of biologically effective UVB (UVB_{eq}) for up to 24 h in our study was close to that found in Arabidopsis exposed for 72 h (Landry et al. 1995), in which evident leaf injury is also observed. The dark repair process at night might be very important and necessary to protect plants against UV stress, and continuous UV exposure might cause more severe injury to plants. In addition, a low level of visible light generally accentuates the injurious effect of UV on plants (Teramura 2006). In our study, the rice seedlings were subjected to low level visible light (20 μmol m^{-2} s^{-1}) during UV exposure. Injured responses strongly indicated the effects of accumulated UV on plants with successive exposure. Rice seedlings experienced exposure from the assimilation stage (6 and 12 h) to the damage (24 h) stage in our work.

Protective responses to UV stress

To defend against the adverse effects of UV, plants have developed protective strategies (Brosché and Strid 2003, Frohnmeyer and Staiger 2003). In the present study, UV-induced strategies in rice seedlings mainly included accumulation of UV-absorbing compounds and enhanced expression of proteins with protective functions (SAMS, glyoxalase I, chitinase, Bet v I allergen family protein and Hsp-related proteins).

Our results suggested that up-regulation of SAMS related to polyamine synthesis and significant accumulation of UV-absorbing compounds might be a basic protective response in rice seedlings during UV exposure. UV-induced secondary metabolites, such as polyamines and UV-absorbing compounds, could directly counteract UV stress or indirectly protect plants via suppression of ROS and reduction of oxidative damage. SAMS catalyzes the formation of S-adenosylmethionine, which is also a precursor of polyamines (Yan et al. 2006). Polyamines may mediate UV stress, possibly through scavenging the radical (Jordan 1996). UV-absorbing compounds can filter 90% of UV through the epidermis (Delucia et al. 1992) and play a role in scavenging free radicals (Rice-Evans et al. 1997). Accumulation of UV-absorbing compounds is considered as the most effective protection strategy in response to UV (Frohnmeyer and Staiger 2003). UV-absorbing compounds increased by 54.6% at the late stage (24 h) in our study, but rice leaves were obviously damaged. The result implied that it was not enough to defend against UV stress only through accumulation of UV-absorbing compounds when UV exposure increased to a particular extent.

The well-known detoxification/antioxidation protein glyoxalase I was induced at the protein level and significantly up-regulated at the mRNA level at 12 and 24 h in our study. This indicated the start of the detoxification/antioxidation process of plants to UV exposure, and glyoxalase I might confer detoxification of methylglyoxal and defense against oxidative damage at the assimilation and damage stages in rice plants. It has also been suggested that glyoxalase I is the most obvious differentially affected protein (increase 15-fold) in the identified proteins in maize plants grown in sunlight, compared with those in sunlight depleted of UVB radiation (Casati et al. 2005). Overexpression of glyoxalase I in transgenic tobacco results in increased detoxification of methylglyoxal, accumulation of glutathione and tolerance to high levels of salinity (Reddy and Sopory 1999, Yadav et al. 2005).

The other induced proteins at 12 or 24 h in our study were defense proteins, chitinase and Bet v I allergen family protein, which might play a key role in constructing a self-defense mechanism together with glyoxalase I. Chitinase (PR-3 family) and Bet v I allergen family protein (PR-10) belong to the PR group of defense proteins (Breiteneder andEbner 2000). PR proteins...
inhibit pathogen development and increase resistance to stress (Bol et al. 1990). UV exposure induces the expression of PR proteins such as PR-1, PDF1.2, PR-2 and PR-5 in Arabidopsis (Surplus et al. 1998, A.-H.-Mackerness et al. 1999, Kalbina and Strid 2006).

The up-regulation of four Hsp-related proteins (Hsp70-related protein, Hsp70 mitochondrial precursor, putative Hsp70 mitochondrial precursor and HspST1) in our study might also be linked to UV stress resistance. Hsp oligomers can recognize and bind to stress-induced proteins with non-native conformations, minimize the aggregation of non-native protein, and remove the non-native or aggregated protein from the cell (Feder and Hofmann 1999). Previous studies also indicate that Hsp-related proteins play a role in response to UV in some species, including rice, soybean and maize (Murakami et al. 2004, Casati et al. 2005, Xu et al. 2008).

**Materials and Methods**

**Plant culture and UV treatment**

*Oryza sativa* L. *indica* cultivar 93-11, which is widely planted in China and in Asia-Pacific regions, was used in this study. Rice seeds were soaked in distilled water for 48 h at 30°C, and then incubated on vermiculite under white fluorescent illumination of 100 μmol m⁻² s⁻¹ in 12 h light/dark cycles at 25°C. Sixteen-day-old seedlings with four leaves were transferred to continuous white fluorescent illumination of 20 μmol m⁻² s⁻¹, supplemented with UV from UV fluorescent lamps (Beijing Institute of Opto-Electronic Technology). Cellulose diacetate film of 0.125 mm thickness (Lucky Films Co. Ltd.) was used to filter UVC (<280 nm) irradiation. The intensity of UVB and UVA wavelengths was measured using a Spectroradiometer 320 (1 nm resolution, Instrument system GmbH). Furthermore, the UVBₚₑ was gained through normalization at 300 nm, and calculation according to Caldwell (1971). The intensities of UVBₚₑ and UVA were 0.67 and 0.28 W m⁻², respectively. The accumulated levels of UVBₚₑ exposure for 6, 12 and 24 h, were 14.4, 28.7 and 57.5 kJ m⁻², respectively. At 6, 12 and 24 h treatment, the fourth leaves of randomly selected seedlings were frozen in liquid nitrogen and then stored at −80°C until the time of assay. The rest of the seedlings were adapted in the dark for 30 min before Chl fluorescence measurements.

**Measurements of physiological parameters**

(Pn, Cond, Fₛ/Fᵣ, Chl content, MDA and UV-absorbing compounds)

Pn and Cond were determined by a portable gas analysis system (LI-COR 6400, Li-Cor Inc.), at a saturating photosynthetic photon flux density (PPFD) of 1,000 μmol m⁻² s⁻¹, temperature of 25°C and CO₂ concentration of 400 μmol mol⁻¹. The maximum photochemical efficiency of PSII (Fₛ/Fᵣ) was measured using a PAM 101 Chl fluorometer (Heinz Walz GmbH). The minimal fluorescence level, F₀, was determined with a <1 μmol m⁻² s⁻¹ measuring modulated light, which was low enough to avoid inducing significant variable fluorescence. The maximal fluorescence level, Fₘ, was determined by a 0.8 s saturating pulse at 8,000 μmol m⁻² s⁻¹. The maximum Chl fluorescence ratio, Fₛ/Fᵣ, was referred to (Fₛ/Fᵣ)/Fᵣ. Chl (0.2 g FW) was extracted from leaves in 95% ethanol in the dark for 48 h at 4°C. The extract was then assayed according to Arnon (1949).

Lipid peroxidation was determined by measuring the amount of MDA. Leaf samples (0.2 g FW) were quickly homogenized with a pestle in a chilled glass mortar with 2 ml of 5% (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 15,000 × g for 20 min at 4°C. The supernatant (1 ml) was transferred into 5 ml of 0.67% thiobarbituric acid, heated at 95°C for 30 min, and then cooled immediately in an ice bath. The mixture was centrifuged again, and the absorbance was read at 532 and 600 nm with a Shimatzu UV-300 spectrophotometer (Shimatzu Co. Ltd.). The MDA concentration was calculated using the extinction coefficient 155 mM⁻¹ cm⁻¹.

UV-absorbing compounds were extracted through pre-cooled 70% methanol containing 1% HCl at 4°C for 2 h (2 ml per 80 mg FW). The mixture was centrifuged at 1,800 × g for 15 min. The amount of UV-absorbing compounds (the absorbance at 330 nm per mg FW) was measured at 330 nm with a Shimatzu UV-300 spectrophotometer.

Six leaves for each sample were measured for Pn, Cond and Fₛ/Fᵣ. Three independent replicated experiments were conducted for the control of Chl, MDA and UV-absorptive compounds. Student’s t-test was performed using SPSS 13.0. Differences between treatments were considered significant if P < 0.05.

**Protein extraction**

A 200 mg frozen leaf sample was ground in liquid nitrogen, and then suspended in pre-cooled 10% (w/v) TCA/acetone with 0.1% dithiothreitol (DTT) for 2 h at −20°C, followed by centrifugation at 35,000 × g for 15 min at 4°C. Precipitates were washed with pre-cooled acetone and 0.1% DTT three times, centrifuged and dried. The dried protein powder of each sample was dissolved in lysis buffer containing 9 M urea, 4% CHAPS, 2% IPG buffer (pH 4–7), 1% DTT and 20 mM Tris–HCl pH 8.5 for 12 h. The protein concentration was measured using the Bradford method. The solution was stored in 1.5 ml aliquots at −70°C. Proteins were extracted three times from each sample.

**2-DGE**

Leaf protein solution (1 mg per gel) was mixed with rehydration buffer containing 9 M urea, 2% CHAPS, 0.5% IPG buffer (pH 4–7), 1% DTT and 0.002% bromophenol blue, and rehydrated with 18 cm (pH 3–10, linear) IPG strips for 12 h in a re-swelling tray at 20°C. After rehydration, the strips were placed in an Etan IPGphor Cup Loading Manifold (GE Healthcare). Isoelectric focusing was carried out at 20°C with Etan IPGphor II until the Volt-hours value reached 50,000 Vh. The
strips were equilibrated twice (Salekdeh et al. 2002). SDS–PAGE in the second dimension was performed in 15% separation gels using an Etan Dalt II electrophoresis system (Amersham Biosciences) at 5 W per gel for 45 min followed by 20 W per gel for 3 h. Separated protein spots were visualized using Coomassie Brilliant Blue R-250. Stained gels were scanned using a UMAX Power Look 2100XL scanner (Maxium Tech.). Image analysis was accomplished with ImageMaster™ 2D Platinum software Version 5.0 (Amersham Biosciences). The optimized parameters were: Smooth, 1; Min Area, 5; and saliency, 8. After automated detection and matching, manual editing was conducted. Spot intensity is given as the mean value of spot volumes from three biological replicates. The spot volumes between treatment and control were analyzed by Student’s t-test. The relative ratio of the spot intensity between treatment and control is represented by fold. The threshold for significance was set at $P < 0.05$ and >1.5-fold (treatment/control) or less than −1.5 fold (control/treatment) (Casati et al. 2005).

**Protein spots identification**

Gel spots were carefully excised from two-dimensional gels, destained with acetonitrile, reduced with 10 mM DTT in 25 mM ammonium bicarbonate, alkylated with 55 mM iodoacetamide in 25 mM ammonium bicarbonate, and washed with 25 mM ammonium bicarbonate in water/acetonitrile (50/50). Spots were then dried in a SpeedVac and digested overnight at 37°C in modified trypsin solution (1 ng ml$^{-1}$ in 25 mM ammonium bicarbonate). The reaction was stopped by adding 2 ml of 10% trifluoroacetic acid (TFA). The digestion was desalted with Poros R2, and then eluted with $\alpha$-cyano-4-hydroxycinnamic acid (12 mg ml$^{-1}$) in 70% acetonitrile with 0.1% TFA. The eluted solution was placed into a target well, dried and analyzed using a Bruker AutoFlex MALDI-TOF mass spectrometer. The instrument was operated in the reflectron mode under 19 kV accelerating voltage and an m/z range of 600–4,000. Monoisotopic masses were processed for identification through analysis with m/z software and search in the NCBInr database using MASCOT (Matrix Science).

**qPCR**

qPCR analysis was used to detect mRNA expression patterns of genes encoding tryptophan synthase $\alpha$ chain (spot 3), glyoxalase I (spot 5) and Bet v 1 allergen family protein (spot 23). RNA was extracted from leaf samples at three different time points (6, 12 and 24 h) by the Trizol (Invitrogen) method. Reverse transcription was performed using M-MLV reverse transcriptase (TAKARA Code: D2639A) according to the manufacturer’s protocol. Real-time PCRs were conducted using gene-specific primers (Table 2) on a 7300 Real-Time PCR system (Applied Biosystems) using SYBR® Premix Ex Taq™ (TAKARA Code: DRR041A). Actin was used as a reference gene. The relative quantifications of mRNA expression were calculated using the comparative cycle threshold method (Livak and Schmittgen 2001), and analyzed by Student’s t-test comparing treated and control samples from three biological replicates.

**Supplementary data**

Supplementary data are available at PCP online.

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**References**


### Table 2 Sequences of the primers used in qPCR

<table>
<thead>
<tr>
<th>Protein</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tbody>
<tr>
<td>Spot 3</td>
<td>CTCACAGGACAGGGGAGACCCG</td>
<td>CTCACAGGACAGGGGAGACCCG</td>
</tr>
<tr>
<td>Spot 5</td>
<td>GGACGAGGATTTCGGGAGGCCTGCG</td>
<td>GGACGAGGATTTCGGGAGGCCTGCG</td>
</tr>
<tr>
<td>Spot 23</td>
<td>TGTCACCGACATGACCGTCAACCC</td>
<td>ATGCGCAAGTACGCGTCCGAGAT</td>
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<tr>
<td>Actin</td>
<td>CTCATAGGAAATGGAAGCTGCGGGTA</td>
<td>CGACCCACCTTGATCTCTGCTGTA</td>
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</table>

The spot number is consistent with spot numbers in Fig. 3.

Supplementary data are available at PCP online.


