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REVIEW

Proteomics characteristics of rice leaves in response to environmental factors

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Abstract Rice is an important food crop worldwide. Its productivity has been influenced by various abiotic and biotic factors including temperature, drought, salt, microbe, ozone, hormone and glyphosate. The responses of plants to stress are regulated by multiple signaling pathways, and the mechanisms of leaf growth and development in response to stress remain unclear to date. Recently, proteomics studies have provided new evidence for better understanding the mechanisms. The proteins in response to different stress conditions are mainly involved in photosynthesis, signal transduction, transcription, protein synthesis and destination, defense response, cytoskeleton, energy, cell wall and other metabolism. In addition, some stress type-specific proteins have been identified, such as small heat shock proteins under temperature stress, S-like RNase homolog and actin depolymerizing factor under drought stress, ascorbate peroxidase and lipid peroxidation under salt stress, probenazole-inducible protein and rice pathogenesis-related proteins under blast fungus. Many of the proteins including ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO), molecular chaperones, antioxidases and S-adenosylmethionine synthetase play very important roles in leaves. This paper reviews the proteomic characterization of rice leaves in response to various environmental factors.

Keywords rice, leaf, proteomics, environmental factor

1 Introduction

Rice (*Oryza sativa* L.) is a primary food source for more than half of the human population in the world. The completed genome sequences for *O. sativa* L. ssp. *indica* (Yu et al., 2002) and *O. sativa* L. ssp. *japonica* (Goff et al., 2002) have provided a rich resource to study global protein expression profiles in any given tissue, such as leaf, leaf sheath, stem, root, seed, and pollen from rice (Zhong et al., 1997; Koller et al., 2002; Shen et al., 2002; Dai et al., 2006; Nozu et al., 2006; Yang et al., 2007a). Leaf is a very important organ due to its functions of photosynthesis, respiration, and photo-perception that are directly related to the rice yield. A large number of papers on rice leaf proteomics have been published in the last decade (e.g. Koller et al., 2002; Zhao et al., 2005; Yuzo et al., 2006). More than 2000 proteins identified and classified into the following functional categories: cell growth and division, cell rescue and defense, cell death and aging, cellular communication/signal transduction, energy and metabolism, intracellular transport, protein synthesis and destination, and transcription (Koller et al., 2002). Proteins involved in photosynthesis and energy production were most abundant in leaves.

The productivity of rice was clearly influenced by various abiotic and biotic stresses including temperature, drought, salt, microbe, ozone, hormone, and glyphosate. Identification of proteins/genes response to biotic or abiotic stresses is a basic step toward understanding the molecular mechanisms, and thereby producing transgenic plants with enhanced tolerance to a particular stress condition. Some differentially-expressed proteomes in rice leaves under the aforementioned stress conditions have been reported (Hajduch et al., 2001; Agrawal et al., 2002b; Salekdeh et al., 2002; Kim et al., 2004; Kim, 2005; Lee et al., 2007a; Ahsan et al., 2008). Based on these proteomic studies, some important proteins in response to different environmental stresses were identified, such as pathogenesis related (PR) proteins [*Oryza sativa* pathogenesis-related protein 1 (OsPR1), OsPR5, and OsPR10]

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(Agrawal et al., 2002b; Kim et al., 2004), antioxidant enzymes [ascorbate peroxidase (APX) and super oxide dismutase (SOD)] (Parker et al., 2006), osmotic stress-response 26S proteasome regulatory subunit and dnak-type molecular chaperone BiP (Zang and Komatsu, 2007), ozone stress-related ATP-dependent caseinolytic protease (CLP) (Agrawal et al., 2002b), and actin depolymerizing factor for the drought stress (Salekdeh et al., 2002). These findings have enabled us to better understand and further investigate the mechanisms of rice in response to environmental factors. This review synoptically describes proteomics compilation in the rice leaves in response to various environmental stimuli.

2 Proteome characteristics of rice leaves in response to environmental factors

2.1 Temperature

Temperature stress has widely affected crop cultivation and productivity. Heat can be rapidly sensed by various biomolecules in the plasma membrane, cytosol, and subcellular organelles of cells, and provokes molecular signals for the heat shock response. Generally, there is a significant reduction in transcription and translation processes in response to heat (Kim et al., 2002). On the other hand, cold stress can also lead to dramatic changes in gene expression, biomembrane lipid composition, and small molecule accumulation in leaves (Thomashow, 2001).

When rice leaves from 2-week-old seedlings were subjected to 42°C for 12 h and 24 h, respectively, the relative ion leakage and lipid peroxidation were increased. This suggests that rice leaves exposed to high temperature frequently experience oxidative stress. Approximately 73 protein spots were differentially expressed at least at one time point. A total of 48 identified heat-responsive proteins include heat shock proteins (HSPs) [such as HSP70, dnak-type molecular chaperone BiP, chaperonin 60 (Cpn60) beta precursor, and mitochondrial-targeted small heat shock protein (sHSP)], redox proteins [such as glutathione S-transferase (GST), dehydroascorbate reductase (DHAR), and thioredoxin *h*], regulatory proteins (such as chloroplast elongation factor Tu, cysteine proteinase), energy and metabolism proteins [such as transketolase, uridine diphosphate (UDP)-glucose pyrophosphorylase and thiamine biosynthesis protein]. Among them, the expression of a group of HSPs and the proteins for energy metabolisms changed significantly under heat stress. This indicates that energy and metabolic pathways are highly disturbed and HSPs may play a pivotal role in protecting cells from damaging under heat stress (Lee et al., 2007a). Moreover, a similar change of proteome was found in 7-day-old seedlings exposed to 35°C, 40°C, and 45°C for 48 h, respectively (Han et al., 2009). The differentially-expressed proteome in response to a series of high temperature stresses proved that rice seedlings probably adopted different strategies to cope with various temperature stresses. The higher the temperature, the more protection machineries were involved. Below 35°C, photosynthetic capability-related mechanisms in seedlings are activated, and certain antioxidative pathways begin to be active when a high-temperature stress at or above 40°C. Once seedlings suffer an intense high-temperature stress at 45°C, HSP-related protection mechanisms are induced, besides the antioxidative pathways and photosynthesis protection processes (Han et al., 2009). The high temperature-responsive proteome in *Oryza meridionalis* Ng. (a wild relative species of *O. sativa* with high temperature tolerance capability) also exhibited that the enzymes involved in calvin cycle, photosynthetic electron transport, dark reaction of photosynthesis, and HSPs were over-representative in these heat-responsive proteins (Scafaro et al., 2010). This suggests a specific connection between photosynthetic enzymes and temperature stress in rice.

Low temperature stress is one of the most serious environmental stresses affecting plant growth. The proteomics studies of rice leaves under cold stress revealed various cold responsive proteins in signal transduction, RNA processing, transcription, protein synthesis and destination, stress defense, antioxidative/detoxifying reaction, cell wall metabolism, energy pathway and metabolism (Yan et al., 2006). The major subgroup of cold responsive proteins belongs to protein metabolism including 60 kDa chaperonin alpha and beta subunits, 20 kDa chaperonin, HSP70, ATP binding subunit of ATP-dependent CLP, and Ftsh-like protein Pftf precursor. The minor subgroup is involved in the biosynthesis of cell wall components. Additionally, some differentially expressed proteins are responsible for the synthesis of methionine and s-adenosyl methionine (SAM), formation of UDP-glucose, and oxygen evolving reaction of photosynthesis. Among them, 44% of the up-regulated proteins are located in the chloroplasts, implying chloroplasts being one of the organelles inside the cells mostly affected by cold stress (Cui et al., 2005).

Different regulation systems in leaf blades, leaf sheaths, and roots were revealed by the differentially expressed proteins identified in these organs from 2-week-old rice seedlings that were exposed to 5°C for 48 h (Hashimoto and Komatsu, 2007). Most of the differentially expressed proteins were similar in leaf blades and sheaths, but different in roots. However, UDP-glucose pyrophosphorylase was up-regulated in roots and leaf blades, but down-regulated in leaf sheaths. This suggests that complex systems in response to stress exist in plants with organ-specific mechanisms that regulate both the timing and the sensitivity of the response. Lee et al. (2007b) identified

some novel leaf proteins in response to cold stress (5°C or 10°C) in a time-course experiment, such as cysteine proteinase, thioredoxin peroxidase, and a fibrillin-like protein. Among them, a RING (a really interesting new gene) zinc finger protein-like protein is highly responsive to extreme temperature. The RING finger motif is a protein-protein interaction domain that has been implicated in a range of diverse biological processes (Borden and Freemont, 1996). Thus, they speculated that RING/zinc finger domain containing proteins may be used as marker proteins for extreme cold temperature (Lee et al., 2007b).

2.2 Drought

When plants are subjected to drought stress, the leaves lose water and the capability of fixing CO_2 in the photosynthetic apparatus. Drought stress leads to an overall reduction of the photosynthetic electron transport chain (Osmond and Grace, 1995) that induces oxidative stress (Borsani et al., 2001). In addition, the glycolate oxidase pathway that produces H_2O_2 is activated during drought stress (Mittler and Zilinskas, 1994). Salekdeh et al. (2002) investigated the proteomics changes in two rice cultivars (*O. sativa* L. cv CT9993 and cv IR62266) upon drought and rewatering. Forty-two proteins out of over 1000 leaf proteins examined were found to be significantly changed in abundance under stress, and 27 of them exhibited different response patterns in the two cultivars. These proteins have functions in photosynthesis, carbon metabolism, and oxidative stress tolerance. All the changed abundances of the differentially expressed proteins under drought stress were reversed fully or substantially after rewatering. Moreover, four novel drought-responsive proteins were revealed in this work, including up-regulated S-like RNase homologue, actin depolymerizing factor, and RuBisCO activase, and down-regulated isoflavone reductase-like protein. The results showed that a variety of normal physiological activities slowed down, but the activities of antioxidant-related proteins were enhanced, which might be required to adjust cellular response to drought. Some of these differentially expressed proteins were also revealed in recent studies on 2-week-old seedlings under 4 d water deficient stress, such as down-regulated Rieske Fe-S precursor protein and up-regulated chloroplast Cu-Zn SOD (Ke et al., 2009).

2.3 Salt

Salinity is a major abiotic stress affecting plant life processes. Proteomic studies on salinity stress response in rice have been carried out using roots, young panicles, leaf sheaths of rice seedlings under salinity stress varying from 50–150 mmol/L NaCl (Abbasi and Komatsu, 2004; Kim, 2005; Yan et al., 2005; Dooki et al., 2006; Parker et al., 2006). For example, Kim (2005) investigated the physiological and biochemical responses of the fully expanded 3rd leaves of 18-day-old seedlings that were treated for 4 d with 130 mmol/L NaCl. They found 55 abundance-changed spots on 2-dimentional (2D) gels and identified 33 proteins by liquid chromatography-tandem mass spectrometry/mass spectrometry (LC-MS/MS). Most of the identified proteins are involved in photosynthetic CO_2 assimilation and photorespiration. Besides, the key marker enzymes associated with oxidative damage showed remarkable responses to salt stress, such as the induced APX and lipid peroxidase, and the suppressed catalase. Interestingly, ribulose-1, RuBisCO activase, which is capable of activating RuBisCO, was markedly enhanced by salt stress. Another work on salt-responsive proteins in rice showed that the changes of some proteins occur in a time-dependent manner (Parker et al., 2006). When 2-week-old and 20-day-old seedlings were treated with 50 mmol/L NaCl for 7 d and 1 d, respectively, the abundance of some proteins displayed time-dependent expression. A putative phosphoglycerate kinase was found to increase in expression within 24 h and did not increase over a longer period of exposure to salt. However, SOD, S-adenosyl-L-methionine synthetase (SAMS), putative porphobilinogen (PBG) deaminase and translation initiation factor 5A (EIF-5A) showed no changes in 24 h after exposure to salt, but increased or decreased in levels after 7-d salt treatment (Parker et al., 2006).

2.4 Metal ions

Some metal ions are important for normal plant growth and development, such as copper, cadmium, mercury, and strontium. But some heavy metals in soil are strongly toxic to plants. For example, the photosynthetic apparatus is particularly susceptible to cupric ion, which results in a decrease in the electron transfer rates consequent to its binding to numerous sites of photosystem II (Maksymiec, 1997). The other heavy metals like cadmium and zinc are also taken up readily by the plant cells and can be phytotoxic (Chaoui et al., 1997).

Hajduch et al. (2001) studied the patterns of protein changes in the rice leaf in response to heavy metal ions. The middle portions of the mature and fully expanded rice leaves were cut into 3-cm long segments and were floated on 250 μmol/L copper ($CuSO_4$), cadmium ($CdCl_2$), mercury ($HgClO_4$), cobalt ($CoSO_4$), lithium ($LiSO_4$), zinc ($ZnCl_2$), and strontium ($SrCl_2$), respectively. Besides changes in the morphology of the leaf segments (e.g., yellowing symptoms and necrotic lesions), great changes in protein abundance were found by analyzing the protein patterns on 2D gels. Some proteins were induced by heavy metals, e.g., the PR5 and the probenazole-inducible protein 1 (PBZ1). Changes in the major leaf photosynthetic protein, RuBisCO, and its degradation products were significantly higher after metal stress. SOD and oxygen evolving protein were also found to be induced by the

heavy metals. Similar effects on photosynthesis were also found in 10 d-seedlings treated by 100 mmol/L $CdCl_2$ for 24 h, represented by the induction of Photosystem II oxygen-evolving complex (OEC) protein 2 and RuBisCO activase (Lee et al., 2010). Besides, ferredoxin-NADP(H) oxidoreductase and peroxidase (POX) were up-regulated (Lee et al., 2010). Moreover, POX isoenzyme (a detoxification-related protein) was markedly induced in rice seedlings in response to 3-d and 7-d treatments of 0.1 mmol/L $CdCl_2$ (Ge et al., 2009). Thus, we can suspect that POX and photosynthesis-related proteins may play a role in providing metabolic energy and redox power for Cd detoxification.

The photosynthetic mechanism and energy-associated enzymes in rice leaf were also obviously influenced by arsenate (As). When 2-week-old seedlings were subjected to a new group of nutrient solutions containing different doses (50 or 100 μmol/L) of As for 4 d, RuBisCO large subunit (LSU), chloroplast 29 kDa ribonucleoproteins were significantly down-regulated, but some energy-related enzymes, such as NADP-dependent malic enzyme, dihydrolipoamide dehydrogenase, aspartate aminotransferase, NAD dependent formate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase (GADPH) and ATP-dependent protease proteolytic subunit ClpP-like protein, were up-regulated (Ahsan et al., 2010). This indicates that the photosynthetic mechanism was damaged, but higher energy was required for activation of the metabolic processes in leaves exposed to As.

2.5 Microbe

In order to resist incompatible pathogens, plants depend on timely recognition of the invading pathogen and rapid activation of defense responses via a number of signal transduction and metabolism pathways. For example, differential expression of proteins related to signal transduction, antioxidant defense, photosynthesis, metabolism, and protein turnover were detected in rice during the *Xoo* infection by using proteomics approaches (Yu et al., 2008). Of the many plant defense responses to pathogen invasion, the mostly studied is the expression of a group of host-encoded proteins referred to as PR proteins, which occur in a wide variety of plant species and whose expression is governed by a cascade of signal transduction. The developmental stage of the plant is one of the important factors that determine the types of PR to be induced. In the past years, there were many proteomics studies using leaf blades of rice plants infected with blast fungus *Magnaporthe grisea* (Kachroo et al., 1997; Konishi et al., 2001; Kim et al., 2004). After 24, 48, and 72 h of inoculation, eight proteins resolved on the 2D gels were found to be induced or increased in the inoculated leaves. They were two receptor-like kinases (RLKs), two beta-1.3-glucanases (Glu1 and Glu2), thaumatin-like protein (TLP), POX 22.3, PBZ1, and OsPR10. Interestingly, these proteins were reported to be also induced by jasmonic acid (JA). Probably, the early and high induction of these proteins under blast fungus stress may prepare host plants to defend themselves (Kim et al., 2004). Besides, rice blast fungus and JA might utilize common mechanisms for transducing signals in activating pathogen responsive proteins in rice.

2.6 Hormone

Plant hormones such as brassinosteroids (BRs), abscisic acid (ABA), and JA are important in plant growth and development including seed germination, stem elongation, leaf expansion, as well as flowering and fruit development. The application of brassinolide (BL) to the lamina joint region of rice (*O. sativa* L. cv. Nipponbare) seedlings was investigated by Konishi and Komatsu (2003). The proteins related to photosynthesis and stress tolerance were mainly found in the lamina joints and roots, respectively. In their study, lamina joint segments, excised from 8-day-old seedlings, were floated on 1 μmol/L BL and 10 μmol/L ethephon and incubated under continuous light at 25°C for 48 h, respectively. It appeared that there was more degradation of RuBisCO (which resulted in lamina inclination) than controls without hormones. In addition, two homologous proteins of GST increased in both the lamina joints and roots after BL application. Plant GSTs attach glutathione to electrophilic xenobiotics, which tags them for vacuolar sequestration (Edwards et al., 2000) and are essential for the conjugation and transport of specific metabolites. It suggests that GST plays a role in the functions of BL, and the physiological functions are implicated in rice lamina inclination and root elongation triggered by BL (Konishi and Komatsu, 2003).

Rakwal and Komatsu (2004) presented the first systematic study on the changes of protein patterns in leaf and leaf sheath after treatment with 5 or 50 μmol/L ABA. Amino acid sequence analysis of affected proteins revealed that ABA caused drastic changes in major photosynthetic proteins [RuBisCO and oxygen-evolving enhancer (OEE) proteins] and certain defense/stress-related proteins, such as manganese superoxide dismutase (MnSOD) and fructose-bisphosphate aldolase. He and Li (2008) immersed the roots of 2-week-old seedlings in a 50 μmol/L ABA solution for 1, 3, or 6 h, respectively. Among the differentially expressed proteins, phosphorylation of six proteins was induced/increased by ABA, including two up-regulated proteins (G protein beta subunit-like protein and glyoxysomal malate dehydrogenase), and four down-regulated proteins (APX, MnSOD, triosephosphate isomerase, and putative Ca^{2+}/H^{+} antiporter regulator protein). Moreover, the increased RuBisCO fragments and changes in some protein phosphorylation status indicate that post-transcriptional modification process is induced by ABA.

In order to study the role of JA in regulating the rice self-defense, Mahmood et al. (2007) treated rice seedlings with

10 μmol/L and 100 μmol/L JA for 1, 2, and 3 d, respectively. They detected 12 up-regulated proteins and 2 down-regulated proteins in cytosolic and membrane fractions. These proteins were categorized into metabolism (such as DNA terminal protein, GADPH, ATP dependent CLP, RNase S-like protein, and glycine rich protein), energy (RuBisCO activase, RuBisCO subunit binding protein α subunit, and RuBisCO LSU), and defense related proteins (PR5 and PBZ1). The level of GADPH was up-regulated after JA treatment, which indicates that changes in metabolic proteins played an important role in JA mediated processes. RNase S-like protein was up-regulated by JA, suggesting its role in the disease resistance or abiotic stress-tolerance through hypersensitive cell death in rice. However, the level of glycine rich protein was down-regulated by JA, suggesting that JA-mediated resistance negatively regulates the glycine rich proteins in the defense process of rice. Among the up-regulated proteins, defense related proteins were important ones down-stream of JA signaling in rice. For instance, PR5 and PBZ1 were significantly changed. These two proteins have been proved to be induced by *Magnaporthe griesea* and *Xanthomonas*, and probably have important roles in resistance (Konishi et al., 2001; Mahmood et al., 2007).

2.7 Toxic levels of atmospheric gases

Ozone (O_3) and CO_2 levels can significantly affect photosynthesis, growth, and yield. A number of studies were conducted at physiological levels to gain an understanding of the processes, whereby plants respond and adapt to the elevated O_3 and CO_2 levels. Agrawal et al. (2002b) examined the effect of O_3 on 2-week-old rice seedlings. Out of a total of 56 proteins investigated, 36 proteins were N-terminally and one was internally sequenced. O_3 caused drastic reduction in major photosynthetic proteins and induction of various defense/stress related proteins. Some protein accumulations were prominently induced by 24-h O_3 treatment, including five PR5 proteins, three PR10 proteins, APX, SOD, calcium-binding protein, calreticulin, a novel ATP-dependent CLP. Some specific and rapidly accumulated proteins, such as OsPR5, OsPR10, APX, MnSOD, and an ATP-dependent CLP, could serve as potent marker proteins to monitor O_3 related damage in rice, and in plants in general. Similar results were found in 2-week-old seedlings exposed to O_3. The photosynthesis-related RuBisCO LSU, RuBisCO activase, OEE proteins 1 and 2, and proteins involved in energy metabolism (such as ructose-bisphosphate aldolase, chloroplase P, and ATP synthase beta subunit) were obviously affected by O_3. These results indicate that O_3 drastically affects energy metabolism, thus affecting the Calvin cycle, and finally decreasing the photosynthetic rate, total photosynthetic production, and hence final yield (Feng et al., 2008). Furthermore, Cho and coworkers (2008) exposed 2-week-old rice plants to 0.2 ppm O_3 for proteomics analysis at 0, 1, 12, and 24 h, respectively. They found the accumulation of amino acids, gamma-aminobutyric acid, and glutathione in O_3 exposed leaves. This systematic survey showed that O_3 triggered a chain reaction of altered gene, protein, and metabolite expression in multiple cellular processes in rice. In order to investigate the response to higher CO_2 levels, ten-day-old seedlings were exposed to high CO_2 of 760, 1140, and 1520 ppm for 24 h, respectively. Seventy-six spots showed differential expression patterns on 2D gels. Most of the proteins belonged to photosynthesis, carbon metabolism, and energy pathways. Several molecular chaperones and APXs were found to respond to higher CO_2 levels. Concomitant with the down-regulation of photosynthesis rate and stomatal conductance, the levels of enzymes of the regeneration phase of the Calvin cycle were decreased. From this, it can be seen that proteins showing maximal changes contribute to the corresponding physiological state of rice in response to CO_2 (Bokhari et al., 2007).

2.8 Glyphosate

Glyphosate is one of the most widely used herbicides in cereal-growing regions worldwide. Ahsan et al. (2008) analyzed the protein expression profiles in rice leaves exposed to glyphosate in order to investigate the effects of glyphosate. A total of 25 differentially expressed proteins were identified, wherein 18 proteins were up-regulated and 7 down-regulated. These proteins had shown parallel expression patterns in response to paraquat. RuBisCO LSU was significantly decreased by the treatment of both herbicides, together with the appearance of visible foliar injury caused by the herbicides. Additionally, an increased accumulation of antioxidant enzymes, including APX, GST, thioredoxin h-type, nucleoside diphosphate kinase 1, peroxiredoxin, and a Cu-Zn SOD chloroplast precursor in the glyphosate-treated sample suggested that glyphosate treatment possibly induced oxidative stress in plants.

3 Key stress responsive proteins in leaves revealed by proteomics

3.1 RuBisCO and its activase

RuBisCO, including LSU and small subunit (SSU), are the most abundant soluble proteins susceptible to environmental factors. They play key roles not only in photosynthetic carbon assimilation, but also in participating in certain signaling cascades. Except for the up-regulation of RuBisCO LSU in response to wounding (Shen et al., 2003) and salt (Kim, 2005), the expression of RuBisCO LSU shows down-regulation in response to cold (Yan et al., 2006), metal (Hajduch et al., 2001), BL (Konishi and Komatsu, 2003), ABA (Rakwal and

Komatsu, 2004), JA (Mahmood et al., 2007), ozone (Agrawal et al., 2002b), and herbicide (Ahsan et al., 2008). Probably, RuBisCO is highly susceptible to a number of exogenous, endogenous, and even chloroplastic proteases, such as trypsin, chymotrypsin, proteinase K, and papain. A recent hypothesis is that reactive oxygen species (ROS) may modify RuBisCO, facilitating its subsequent degradation by proteases (Desimone et al., 1996). It was noticed that the fragmentation of RuBisCO caused by ROS was specific to the LSU. The degradation of RuBisCO LSU may be a possible molecular response of decreased amounts of chlorophyll and the net photosynthetic rate, and the acceleration of leaf senescence under stress.

In vivo, the activation and maintenance of RuBisCO activity are facilitated by RuBisCO activase. RuBisCO activase is an ATP-dependent enzyme and functions to remove sugar phosphates from RuBisCO catalytic sites to facilitate CO_2 binding (Portis, 2003). Thus, the expression abundance and activity of RuBisCO activase ultimately determines the proportion of available RuBisCO active sites. RuBisCO activase is conserved in photosynthetic organisms across a wide range of genera, including lower eukaryotes. Increased activase activity may be required to tolerate salt stress due to a direct reduction in stomatal conductance and subsequent low CO_2 levels. Low stromal CO_2 will result in increased rates of RuBisCO inactivation through the binding of inhibitory sugars prior to carboxylation. Increases in stromal levels of the large isoform of the activase may directly allow carboxylation to occur at low CO_2 levels. Similarly, the accumulation of RuBisCO could reflect the increase of photorespiration under salt stress (Kim, 2005). In rice leaves, RuBisCO activase shows up-regulation under drought (Salekdeh et al., 2002), salt (Kim, 2005), and JA stress (Mahmood et al., 2007). A RuBisCO activase precursor fragment was also induced by copper, cadmium, mercury, lithium and zinc, but remained unaffected by cobalt and strontium treatment (Hajduch et al., 2001). These results strongly suggest damaging effects on the photosynthetic apparatus under these stress and the activity of RuBisCO activase increased in response to the stress.

3.2 Molecular chaperones

Noticeably, molecular chaperones are thought to prevent aggregation, repair the stress-damaged proteins, and aid their renaturation, in addition to protecting cells from the effects of stress. Besides heat stress, the expression levels of molecular chaperones changed in response to a large number of other stresses such as heavy metals (Hajduch et al., 2001), hormones (Konishi and Komatsu, 2003), chilling (Cui et al., 2005), ozone (Cho et al., 2008), CO_2 (Bokhari et al., 2007), osmotic (Zang and Komatsu, 2007), and herbicide (Ahsan et al., 2008). Precise and complex models of regulation exist in different stress conditions because induction or inhibition of the expression of molecular chaperones was observed in different stress environments. For example, a group of HSPs is highly up-regulated by heat (Lee et al., 2007a) and ozone stress (Cho et al., 2008). The induction of sHSPs suggests that they play a crucial role in combating heat and ozone stress by reestablishing normal protein confirmations and thus, cellular homeostasis. Furthermore, accumulation of chaperone protein, a calreticulin precursor, gradually decreased with increasing concentrations of mannitol. Water deficiency, as a factor of osmotic stress, is expected to lead to increased protein aggregation and denaturation, making increased production of molecular chaperones necessary (Zang and Komatsu, 2007).

3.3 Antioxidases

In rice leaves, the cellular antioxidative system consists of different enzymes, such as SOD, catalase, APX, glutathione peroxidase, and guaiacol-type peroxidase. Accumulation of these enzymes revealed by proteomics in response to various stress has a protective role for normal physiological processes under stress conditions. SOD and APX can be induced by high salinity (Parker et al., 2006), ozone (Agrawal et al., 2002b), herbicide (Ahsan et al., 2008), and be phosphorylated by ABA-induction (He and Li, 2008), suggesting the existence of common stress responsive pathways in the regulation of different stresses in leaf tissue. Meanwhile, SOD can be induced by drought (Salekdeh et al., 2002) and heavy metal (Hajduch et al., 2001), but APX cannot, despite that it can be induced by CO_2 (Borden and Freemont, 1996). This suggests that the inducible expression of some proteins is specific to certain environmental stress.

Interestingly, it seems that there is connection to some degree between heavy metal (Hajduch et al., 2001) and GA_3 (Tanaka et al., 2004), light (Yang et al., 2007b) and salt (Kim, 2005), BL (Konishi and Komatsu, 2003), herbicide (Ahsan et al., 2008), and PBZ (Lin et al., 2008) through oxygen evolving protein, SAMS, and GST, respectively. To begin with, the oxygen evolving enhancer II protein was found to be induced by cobalt, zinc, and strontium, drastically reduced by cadmium and mercury, and remained almost unchanged by copper and lithium. In the case of GA_3, photosystem II oxygen-evolving complex is also induced. Secondly, GSTs have various functions such as detoxifying xenobiotics or endogenous secondary metabolites via conjugation with the tripeptide glutathione (GSH), or in stress metabolism via acting as glutathione peroxidases, antioxidant activity, or signaling (Edwards and Dixon, 2005). In rice, there are at least 59 putative GST genes organized into four main phylogenetic classes (tau, phi, zeta, and theta) (Soranzo et al., 2004). Among them, GSTU17, a tau class GST, was strongly induced by PBZ (Lin et al., 2008). GST also showed a significant up-regulation when exposed to glyphosate, suggesting that plants also utilized the antioxidant defense mechanisms to

protect them from the glyphosate stress (Ahsan et al., 2008). Additionally, GSTs with high affinity for auxins and cytokinin increased with BL treatment (Konishi and Komatsu, 2003). In rice, OsGST2 was induced by blast pathogen (Agrawal et al., 2002a), and a putative GST protein was related to lesion formation by 2D gel analysis (Tsunezuka et al., 2005).

3.4 SAMS

Two-week-old dark-grown rice seedlings were de-etiolated upon exposure to light, which results in an increase of SAMS that is involved in the biosynthesis of the phytohormone ethylene. Ethylene contributes to the phenotypic development of the apical hook in the de-etiolated rice seedlings (Yang et al., 2007b). On the contrary, two isozymes of SAMS were significantly down-regulated 4.3- and 2.0-fold under long-term salt stress, respectively. Meanwhile, the expression of several transcripts encoding SAMSs was also down-regulated in response to salt stress (Kawasaki et al., 2001). SAMSs catalyze the formation of adenosylmethionine (AdoMet) from L-methionine and ATP. AdoMet is an important methyl group donor utilized in most transmethylation reactions. Reduced SAMSs might result in reduced production of the plant hormone ethylene. In rice, salinity stimulated ethylene biosynthesis in tolerant cultivars, but has little effect in sensitive lines (Lutts et al., 1996). In *Arabidopsis*, reduced expression of the ethylene receptors has been reported after exposure to salt stress (Zhao and Schaller, 2004). In these studies, an increase in SAMSs abundance or activity has been shown in association with increased lignification and glycine betaine synthesis. The down-regulation of SAMS observed in rice suggests that these isoforms might not be involved in the biosynthesis of osmolytes or lignin.

4 Conclusion and perspectives

The identification of stress-related proteins implies that the survival of plant under stress conditions can be attributed to changed expression levels of proteins in diverse functional groups including cell rescues, defense, cell death and aging, signal transduction, energy, metabolism, protein synthesis and destination, and photosynthesis. The proteomics results suggest that plants overcome the stress condition by enhancing synthesis of chaperones to maintain normal protein structure, and retaining normal growth based on modulation of photosynthesis, energy and metabolism. However, due to the weakness associated with two-dimensional polyacrylamide gel electrophoresis (2-DE) gel-based proteomics, low abundant proteins were difficult to be resolved. And the lack of research on post-translational modification and protein-protein interaction has limited the advancement in the field. Moreover, some novel techniques and approaches have not been put into practice such as isobaric tags for relative and absolute quantitation (iTRAQ) LC-MS good for comparing multiple samples and conditions, and blue-native gels good for detection of protein complexes. Therefore, improvement in proteome coverage and detailed analysis of transcription factors, protein-protein interaction and protein modifications should be imminent goals toward elucidating molecular mechanisms underlying rice response to various environmental factors.

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