Agricultural Science
Volume 2, Issue 4 (2014), 35-43
ISSN 2291-4471 E-ISSN 2291-448X
Published by Science and Education Centre of North America

Proteomic Responses of Wheat Roots during Enhanced Ultraviolet-B Radiation Stress

Duan Jiang-Yan[1], Niu Wen-Ting[1], & Jia Zhen-Hu[1]*

[1]College of Life Science, Shanxi Normal University, Linfen 041004, P. R. China

*Correspondence: Jia Zhen-Hu, Assistant professor, College of Life Science, Shanxi Normal University, Linfen 041004, P. R. China. E-mail: jiaxueshi@163.com; Tel：+86-357-2053665; Fax: +86-357-2053665. Duan Jiang-Yan (Ph D.), E-mail: jianyanduan@163.com

DOI: 10.12735/as.v2i4p35 URL: http://dx.doi.org/10.12735/as.v2i4p35

Abstract

Enhanced ultraviolet-B (UV-B) radiation is one of the most important abiotic stresses that could influence the growth and physiological traits of plants. Wheat (*Triticum aestivum*) is also affected by present-day enhanced UV-B radiation. As protein metabolism plays an important role in plant adaptation to UV-B radiation, this study was designed to identify UV-B -responsive proteins in wheat roots. Wheat (Lin Fen NO.3) was exposed to UV-B radiation with normal light (group B) and only normal light (group CK) in growth chambers. Roots were harvested at 3d, 6d and 9d after UV-B radiation. Proteins were extracted and separated by two-dimensional gel electrophoresis (2-DE). 15 protein spots were regulated by UV-B radiation at 6d. Among these protein spots, 3 proteins were up-regulated and 10 proteins were down-regulated. 2 proteins were expressed only in CK group. 3 protein spots were identified using mass spectrometry. They were further verified as Ribulose-bisphosphate carboxylase activase; Catalase (CAT); Hypothetical protein. These data support the assumption that UV-B may have a regulatory role besides damaging effects and that an increased UV-B environment will likely increase this regulatory influence of UV-B radiation.

Keywords: wheat roots, Ultraviolet-B radiation, protein spot

1. Introduction

With the depletion of the ozone layer, the atmosphere becomes more transparent to solar UV radiation, mainly in the wavelength range 290-320 nm (UV-B) (Yao, Chu, & Ba, 2010a). It will impose an abiotic environmental stress on crops. The enhanced level of UV-B has induced damage to almost every aspect of the physiology and biochemistry of plants (Zheng *et al*., 2008; Lee & Shiu, 2009). Most studies about the effects of enhanced UV-B on plants are mainly involved in aboveground parts, such as photosynthesis, chlorophyll content, and some physiological and biochemical traits of leaves. However, the root is an important organ of the plant. What's more, the growth and development of roots can affect the aboveground parts of plants directly. Researches have shown that enhanced UV-B radiation can also do harm to the root growth of plants, although UV-B radiation cannot penetrate belowground effectively (Jiang, Hu, & Niu, 2010). Various studies have demonstrated that roots are more sensitive to enhanced UV-B radiation. Proteomes that roots express maybe different from that leaves, grains, even fruits express, due to their different sensitivity to UV-B radiation and unique functions (Xu & Huang, 2008). Wheat, a kind of important

economic crop cultured in the Northern China, is also sensitive to present-day enhanced UV-B radiation, which gets the attention of many researchers.

In recent years, proteomic-based technologies have been successfully applied to the systematic study of the proteomic responses to a wide range of abiotic stresses in many plant species (Xu & Huang, 2008). Advances in high-throughput proteomics helped to address complex biological questions in various species (Ghosh & Xu, 2014). Protein extraction is usually followed by protein separation and identification that can be achieved with the use of two- dimensional electrophoresis (2-DE) (Wittmann-Liebold, Graack, & Pohl, 2006) or liquid chromatography coupled with tandem mass spec-trometry (LC-MS/MS) (Fournier, Gilmore, Martin-Brown, & Washburn, 2007). Research of UV-B radiation effects on the Proteomes of wheat roots is still in the data accumulation phase, even has not been reported. So we focus on proteomics as a tool for the analysis of gene expression of wheat in response to UV-B radiation. In previous study, the proteomic responses of wheat leaves under UV-B radiation have been observed. To further understand the enhanced UV-B radiation mechanisms of wheat, in this paper, we studied the short-term influence of proteomic responses on growth of wheat seedlings subjected to UV-B stress.

2. Materials and Methods

2.1 Plant Culture

Wheat seeds were procured from Shanxi Wheat Research Institute of Agricultural Sciences. Selected healthy seeds were sterilized for 10 min with 0.1% $HgCl_2$ and washed in running tap water for 50 min. After that seeds were washed with distilled water for twice .Then leaving the seeds overnight for incubation at 4 °C. After 10 hours, 30 seeds were kept in Petri (diameter 10 cm) seeds per dish, with distilled water and left in the dark for germination.

2.2 Experimental Design

The germinating seedlings of wheat were divided into normal light group (CK) and enhanced UV-B radiation group (B). The UV-B radiation treatment was carried out with lightening treatment at the same time. The UV-B radiation dose was 10.08 $KJ.m^{-2}.d^{-1}$. Each treatment had 3 replicates. In B group, Supplemental UV-B radiation was provided by UV-B fluorescent lamps (40W, 305 nm, Shanxi, China) mounted in metal frames with minimum shading. The distance from the lamps to the top of plant apex was about 60 cm and kept constant throughout the experiment. Seedlings were irradiated for 8h (from 8:00 to 17:00) daily centered on the solar noon.

2.3 Protein Extraction

Roots were harvested at 3d, 6d and 9d, immediately frozen in liquid nitrogen, and then stored at –80 °C prior to analysis. Extraction of roots protein followed the procedure described by Agrawal and Thelen (2005). A portion (200 mg) of roots was homogenized in 1 mL lysis buffer containing 8 M urea, 2% NP-40, 0.8% ampholine (pH 3.5 to 10), 5% 2-mercaptoethanol and 5% Polyvinyl pyrrolidone-40, and grinded by a glass mortar and pestle on ice packs. The homogenates were centrifuged in a RA-50JS rotor (Kubota, Tokyo, Japan) for 5 min. The supernatant was centrifuged at 30,000 g for 5 min and subjected to electrophoresis. Protein concentration was determined according to Bradford (1976) using a commercial dye reagent (Bio-Rad Laboratories, Hercules, CA, USA) . Bovine serum albumin (BSA) was used as a standard.

2.4 Two-Dimensional PAGE (Abbasi & Komatsu, 2004)

Prepared samples were separated by 2-DE in the first dimension by IEF tube gels (Daiichi pure Chemicals, Tokyo, Japan) and in the second dimension by SDS-PAGE. An IEF tube gel of 11 cm

length and 3mm diameter was prepared. IEF gel solution consisted of 8M urea, 3.5% acrylamide, 2% NP-40, 2% Ampholine (pH 3.5–10.0 and pH 5.0–8.0), ammonium persulfate and TEMED. Electrophoresis was carried out at 200 V for30 min, followed by 400 V for 16 h and 600 V for 1 h. After IEF, SDS-PAGE in the second dimension was performed in 15% polyacrylamide gels and 5% stacking gels. The gels were stained with CBB, and image analysis was performed. 2-DE images were synthesized and the position of individual proteins on gels was evaluated automatically with Image Master 2D Elite software (Amersham Biosciences, Uppsala, Sweden). The pI and Mr of each protein was determined by 2-DE markers (Bio-Rad, Richmond, CA, USA). Image analysis included the following procedures: spot detection, spot measurement, background subtraction, and spot matching. Only spots that were detected on all the four replicate gels were analyzed further. To correct the variability due to staining, the spot volumes were normalized as a percentage of the total volume of all spots on the gel.

2.5 Protein Identification

The gel spots were excised and washed with 30% acetonitrile (ACN) in 50 mM ammonium bicarbonate prior to DTT reduction and iodoacetamide alkylation. Trypsin was used for digestion at 37 °C overnight. The resulting peptides were extra
followed by C18 Ziptip desalting. For the MS analysis, the peptides were mixed with 7 mg ml^{-1} acyano-4-hydroxy-cinnamic acid matrix in a 1:1 ratio and spotted onto a matrix-assisted laser desorption/ionization (MALDI) plate. The peptides were analyzed on a 4800 MALDI TOF/TOF analyzer (Applied Biosystem, Framingham, MA, USA). Mass spectra (m/z 880–3200) were acquired in positive ion reflector mode. The 25 most intense ions were selected for subsequent MS/MS sequencing analysis in 1 kV mode. Protein identification was performed by searching the combined MS and MS/MS spectra against the green plant NCBI database. A local MASCOT search engine (V.1.9) on a GPS (V. 3.5, ABI) server was used. Proteins containing at least two peptides with confidence interval (CI) values no less than 95% were considered as being identified.

2.6 Statistical Analysis

A repeated measurement is given as the mean ± SD. The comparison of differences among the groups was carried out using the Student's test. Significance was defined as $p < 0.05$.

3. Results

3.1 Effects on Wheat Growth under Enhanced UV-B

Figure 1. The wheat of B groups on sixth day

In the B group (see Figure 1), a significant decline in wheat viability was observed. UV-B radiation coarsened the root and made it short. The browning roots had been forming side-bending. Root lignification had restricted its viability. The leaf area and number of stomata of wheat decreased.

3.2 Content of Proteins Extracted on Different Treatment Days

The levels of proteins with molecular weight of 100, 60, 40, 25 and 20 KDa polypeptides of wheat roots after the UV-B radiation on different treatment days were shown in Figure 2. These alterations ranged from 20 KDa molecular weight to 100 KDa. The synthesis of 21- 31KDa polypeptides was increased on the 6 and 9 treatment days. Figure 3 reveals UV-B exposure caused a decrease in protein content on 3-, 6- and 9-days.

The total protein content extracted from CK group was significantly higher than that extracted from B group on sixth day. On the third and ninth days, the total protein content extracted from CK group was higher than that extracted from B group, but they were not significant. Significance was defined as $p < 0.05$.（Figure 4）

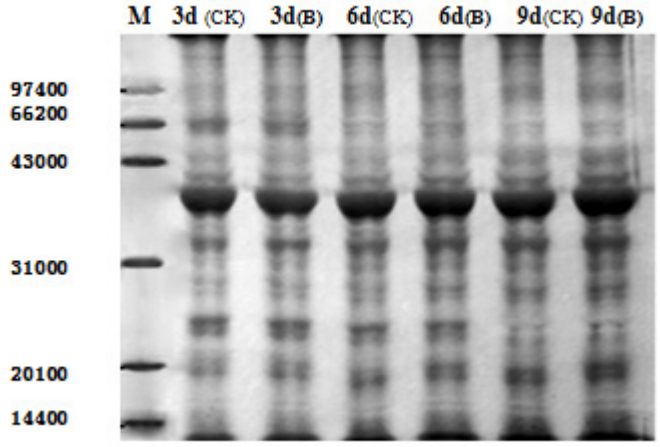

Figure 2. SDS-PAGE of protein in wheat roots after UV-B radiation on 3d, 6d, 9d respectively

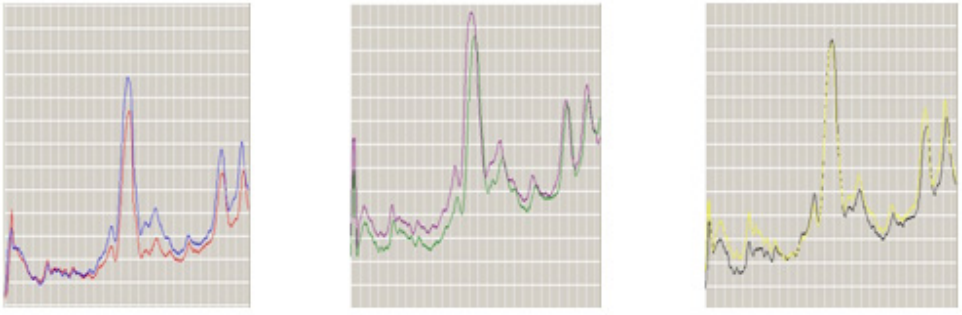

Figure 3. Protein changes observed on the 3d, 6d, 9d between CK and B groups.
The red, green and black curved lines show protein changes of B group.
The other color curved lines show protein changes of CK group.

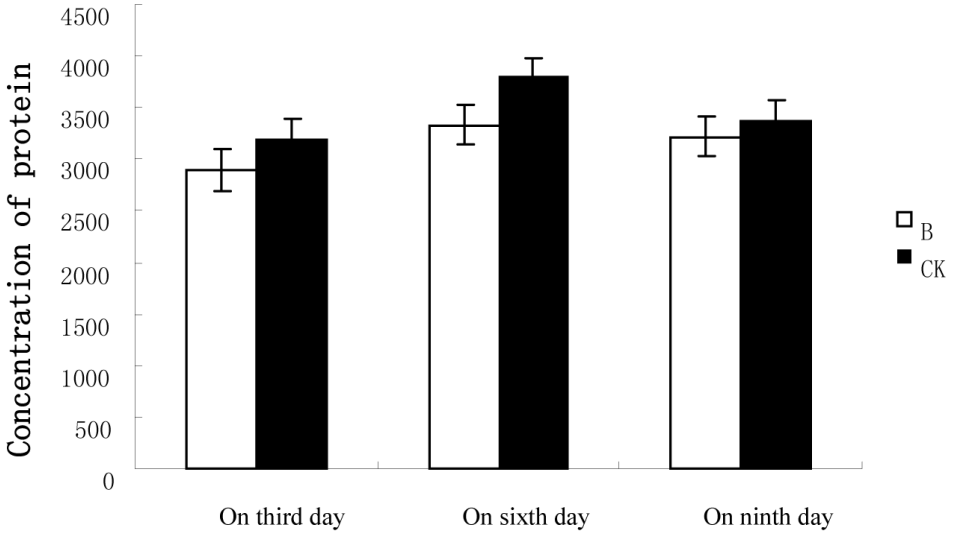

Figure 4. Changes in protein content exposed to different treatment days

3.3 2-DE Gels of Wheat Roots Proteins

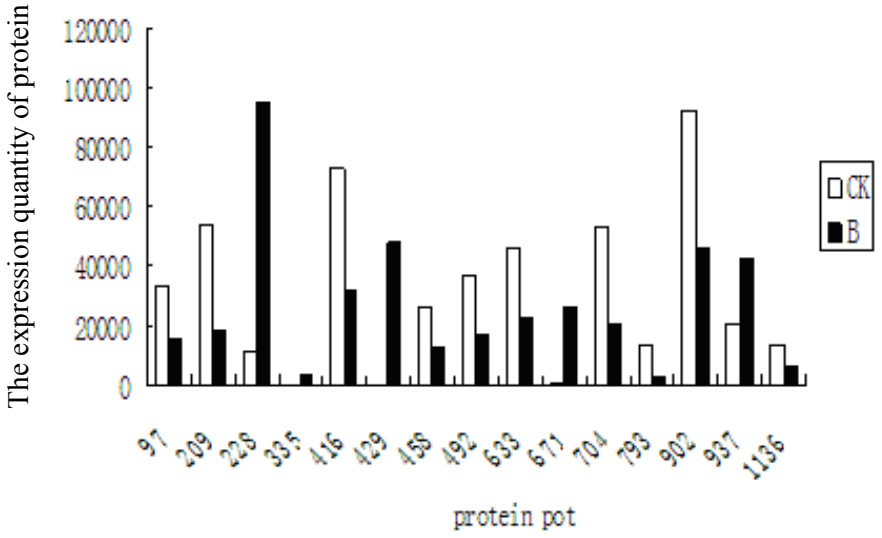

Figure 5. Changes of the differential displayed proteins in wheat roots between B and CK groups

The 2-D polyacrylamide gels were reproducible and exhibited the separated protein spots clearly. Protein spots were significantly affected by UV-B radiation on the six day. There were 15 different protein spots between CK and B groups (Figure 6 & 7). More protein spots exhibited down-regulation than those showing up-regulation under UV-B radiation in B group. Among the 15 protein spots, 3 spots (spots 228，671，937) exhibited increases in intensity or up-regulation in B group, but 10 spots (spots97，209，416，458，492，633，704，793，902，1136) decreases in the intensity or down-regulation. 2 spots (spots 335，429) exhibited only in CK group (Figure 5).

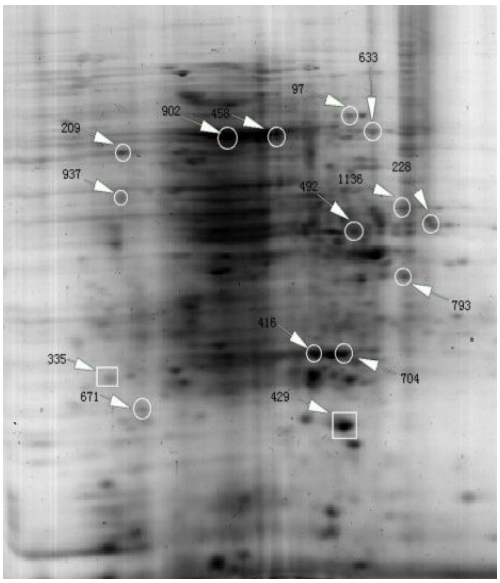

Figure 6. Separation of wheat roots proteins by 2-DE under normal light

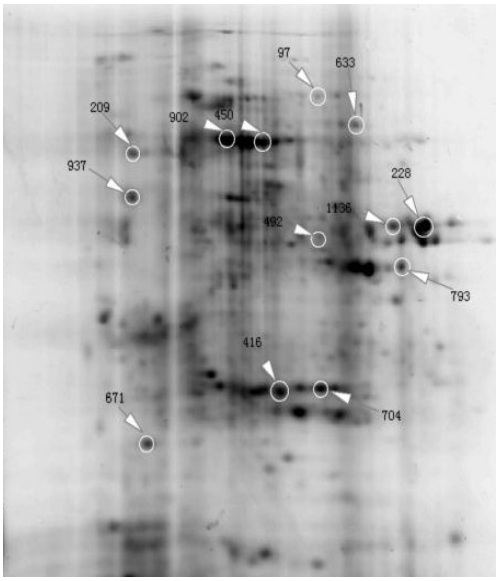

Figure 7. Separation of wheat roots proteins by 2-DE under UV-B radiation

3.4 Analysis of Differential Individual Protein Spots

Three typical protein points were selected for subsequent experiments. The 3 differentially accumulated protein spots were digested with trypsin, subjected to MALDI TOF/TOF MS, and 2 protein spots were identified. Because the statistical data of one spot was under $P \leqslant 0.05$, this protein spot was false positive spot. The results are listed in Table 1.

Table 1. Identification of differential protein spots by ESI-Q MS/MS and database searching

Spot	Protein name	Mr/pI Theoretical Mr/pI	Mr/pI Observed Mr/pI	Score
902	Ribulose-bisphosphate carboxylase activase	24.1/7.3	24.4/7.7	84
937	Catalase(CAT)	247/6.6	248/6.7	87
633	Hypothetical protein	25.7/5.1	23.4/6.3	52

4. Discussion

Enhanced UV-B radiation not only affects aboveground parts but also affects belowground parts of plants (Waring, Underwood, & Baker, 2006). Root is not only vital organs of plants to absorbing salt and water but also is very important for assimilation, synthesis, and translation of many materials. So, root activity directly affected growth and development of plants. At present, enhanced

UV-B radiation is an important abiotic factor that could injure plants by transmittance of UV photons through leaf tissue (Rozema *et al*., 2002). Carbohydrate and energy metabolism could be altered by UV-B radiation, as shown by the up- and down-regulation of several key enzymes of glycolysis, the tricarboxylic acid (TCA) cycle and ATP synthesis. As is evident from the results of this study, Enhanced UV-B injured the wheat seedlings to some extent. The total protein content extracted from CK group was significantly higher than that extracted from B group on sixth day. The protein (spot 902) involved in carbohydrate and energy metabolism. The down-regulation of this protein may contribute to root adaptation to UV-B radiation by lowering respiratory energy consumption. This indicated that wheat is sensitive to root respiration under UV-B radiation.

Active oxygen species play a role in mediating UV-damage. Scavenging of active oxygen and other radical species, through either enzymatic or non-enzymatic systems, can alleviate UV-B stress. In turn, low fluences of UV-B can induce scavenging capacity (Shukla, Joshi, & Kakkar, 2002). Levels of the key antioxidants glutathione and ascorbate are up-regulated in response to UV-B. In order to balance and control the oxygen toxicity, plants have developed antioxidative systems. POD and SOD are important enzymes in plants that protect plants from oxidative damage. Similarly, an increase in antioxidant enzymes' activities of aboveground parts in seedlings subjected to stress condition has also been reported earlier (Yao, Chu, & Ba, 2010b), indicating that up-regulated protein could provide an ecological adaptation for aboveground and belowground parts of seedlings by enhanced antioxidant compounds content and activities of antioxidant enzymes under stress conditions. Interestingly, UV-B also induces lignification. In field studies, lignin accumulation reportedly increases in UV-B-exposed plants. Possibly, lignification is beneficial for the UV-exposed plant (Jbir, Chaibi, Ammar, Jemmali, & Ayadi, 2001). As wheat, a kind of important economic crop cultured in the Northern China, is also sensitive to present-day enhanced UV-B radiation. From this paper, enhanced UV-B radiation caused many up-regulation proteins. The defensive protein (spot 937) was up-regulated in response to UV-B radiation. UV-B radiation coarsened the root and made it short. Root lignification had restricted its viability. Wheat, as one of the most important crops in the world, can alleviate and adapt UV-B stress.

However, in this study, some problems still need to be solved. For example, is there any correlation between the change of total protein in the roots and leaves after enhanced UV-B radiation? If the answer is yes, how the changes of wheat leaves caused by UV-B radiation transfer to the roots? These problems are urgent problems for the next phase of the experiment.

5. Conclusion

In this work we suggest that UV-B may have a regulatory role besides damaging effects and that an increased UV-B environment will likely increase this regulatory influence of UV-B radiation.

Acknowledgements

The study was supported by the Soft Science Foundation of Shanxi Province (2013041038-02) and Natural Science Foundation of Shanxi Normal University (ZK1301).

References

[1] Abbasi, F. M., & Komatsu, S. (2004). A proteomic approach to analyze salt-responsive proteins in rice leaf sheath. *Proteomics, 4*(7), 2072-2081.

[2] Agrawal, G. K., & Thelen, J. J. (2005). Development of a simplified, economical polyacrylamide gel staining protocol for phosphoproteins. *Proteomics, 5*(18), 4684-4688.

[3] Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1-2), 248-254.

[4] Fournier, M. L., Gilmore, J. M., Martin-Brown, S. A., & Washburn, M. P. (2007). Multidimensional separations-based shotgun proteomics. *Chemical Reviews, 107*(8), 3654–3686.

[5] Ghosh, D., & Xu, J. (2014). Abiotic stress responses in plant roots: a proteomics perspective. *Frontiers in Plant Science, 5*(6).

[6] Jbir, N., Chaibi, W., Ammar, S., Jemmali, A., & Ayadi, A. (2001). Root growth and lignification of two wheat species differing in their sensitivity to NaCl, in response to salt stress. *Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie, 324*(9), 863-868.

[7] Jiang, J. Y., Hu, Z. H., & Niu, C. P. (2010). Effects of elevated ultraviolet-B radiation on the chemical composition of wheat straw and the N2O emission from soil amended with the straw. *Ying Yong Sheng Tai Xue Bao, 21*(10), 2715-2720.

[8] Lee, T. M., & Shiu, C. T. (2009). Implications of mycosporine-like amino acid and antioxidant defenses in UV-B radiation tolerance for the algae species Ptercladiella capillacea and Gelidium amansii. *Marine Environmental Research, 67*(1), 8-16.

[9] Rozema, J., Bjorn, L. O., Bornman, J. F., Gaberscik, A., Hader, D. P., Trost, T., . . . Meijkamp, B. B. (2002). The role of UV-B radiation in aquatic and terrestrial ecosystems--An experimental and functional analysis of the evolution of UV-absorbing compounds. *Journal of Photochemistry and Photobiology B: Biology, 66*(1), 2-12.

[10] Shukla, U. C., Joshi, P. C., & Kakkar, P. (2002). Synergistic action of ultraviolet-B radiation and cadmium on the growth of wheat seedlings. *Ecotoxicology and Environmental Safety, 51*(2), 90-96.

[11] Waring, J., Underwood, G. J. C., & Baker, N. R. (2006). Impact of elevated UV-B radiation on photosynthetic electron transport, primary productivity and carbon allocation in estuarine epipelic diatoms. *Plant, Cell & Environment, 29*(4), 521-534.

[12] Wittmann-Liebold, B., Graack, H. R., & Pohl, T. (2006). Two-dimensional gel electrophoresis as tool for proteomics studies in combination with protein identification by mass spectrometry. *Proteomics, 6*(17), 4688–4703.

[13] Xu, C., & Huang, B. (2008). Root proteomic responses to heat stress in two Agrostis grass species contrasting in heat tolerance. *Journal of Experimental Botany, 59*(15), 4183-4194.

[14] Yao, X., Chu, J., & Ba, C. (2010a). Antioxidant responses of wheat seedlings to exogenous selenium supply under enhanced ultraviolet-B. *Biological Trace Element Research, 136*(1), 96-105.

[15] Yao, X., Chu, J., & Ba, C. (2010b). Responses of wheat roots to exogenous selenium supply under enhanced ultraviolet-B. *Biological Trace Element Research, 137*(2), 244-252.

[16] Zheng, L., Su, M., Wu, X., Liu, C., Qu, C., Chen, L., . . . Hong, F. (2008). Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. *Biological Trace Element Research, 121*(1), 69-79.

Copyrights