Pak. J. Bot., 50(6): 2167-2172, 2018.

THE APPLICATION OF EXOGENOUS GIBBERELLIC ACID ENHANCES WHEAT SEEDLINGS UV-B TOLERANCE BY AMELIORATING DNA DAMAGE AND MANIPULATING UV-ABSORBING COMPOUND BIOSYNTHESIS IN WHEAT SEEDLING LEAVES

LIMEI GAO[1*#], XIAOFEI WANG[1], ZHIHUA SHEN[1] AND YONGFENG LI[2#]

[1]*College of Life Science, Shanxi Normal University, Linfen 041004, China*
[2]*Analysis and Testing Center, Shanxi Normal University, Linfen 041004, China*
[*]*Corresponding author's e-mail: limeigao1122@126.com*
[#]*Limei Gao and Yongfeng Li contributed equally to this work.*

Abstract

DNA damage is one of the key parameters to detect UV-B tolerance because DNA is one of the primary targets for UV-B light. Here, we evaluated UV-B stress-induced genomic DNA damages and its self-repair ability in wheat seedlings with or without gibberellic acid (GA_3) treatment. The application of exogenous GA_3 exhibited the better phenotypic development, less inhibition effects in biomass production and a lower accumulation level of cyclobutane pyrimidine dimer (CPD) and (6-4) photoproducts (6-4PPs) in leaves of wheat seedlings under supplementary UV-B stress than those without GA_3 treatment, indicating that GA_3 ameliorated the detrimental effects and DNA damages induced by the enhanced UV-B stress, in which the 150mg L^{-1} of GA_3 is the most effective treatment. After exogenous GA_3 treatment, the UV-absorbing compounds (UACs) contents and DNA photolyase activity also significantly increased, whereas reactive oxygen species (ROS) production were similar between two groups with or without GA_3 application under UV-B stress. These results have demonstrated that GA_3 enhanced wheat seedlings UV-B tolerance by initiating DNA damage repair pathway in leaves of wheat seedlings exposed to supplementary UV-B stress, which were mainly implemented through activating DNA photorepair capability and elevating UV-B absorbance amounts in vivo in plants.

Key words: Gibberellic acid, ultraviolet-B stress, DNA damage repair, wheat.

Introduction

The ultroviolet-B (UV-B) is one of important environmental signal factors that involve the differential regulation of metabolism, development and viability in living organisms (Jenkins, 2009). Prevailing studies have reported that the enhancement of UV-B radiation on the surface of the earth causes diverse stress responses and possibly necrosis in plants, including the damages to nuclear acid, protein and membrane lipids (Jenkins, 2009; Interdonato *et al*., 2011; Monforte *et al*., 2015; Monforte *et al*., 2015; Braun *et al*., 2016). Higher plants have therefore evolved a wide range of mechanisms to protect against UV-B light or repair UV-B damage (Rozema *et al*., 1997). One of the most important protective mechanisms is the repair of DNA damage for plants, such as light-induced repair processes by DNA photolyases (Britt, 2004). In addition, other protective mechanisms are also important, such as the production of cuticular waxes and hairs, the enhancement of cellular antioxidant systems, and the deposition of UV-absorbing phenolic compounds in epidermal tissues (Rozema *et al*., 1997; Brosché & Strid, 2003; Liakopoulos *et al*., 2006).

DNA damages can be induced by many internal or external factors, such as intracellular reactive oxygen species (ROS), UV light radiation, toxic chemicals, and aberrations in cellular processes. The usual types of DNA damage include strand breaks, band cross-links and various base modifications. These forms of DNA damage initiates DNA damage signaling pathway in vivo that regulates a number of physiological processes and phenotypic responses for organisms, including arresting cell cycle progress and promoting DNA repair metabolism (Sancar *et al*., 2004; Jenkins, 2009). In plants, the most common DNA injury included by UV-B exposure is the formation of cyclobutane pyrimidine dimmers (CPDs) and pyrimidine (6-4) pyrimidinone adducts (6-4 photoproducts, 6-4PPs) (Li *et al*., 2004). Of course, previous studies have showed that both types of DNA damage can be repaired by photoreactivation (in the presence of 360-420nm of UV-A/blue light) reaction and photorepair (light-dependent processes) pathway via DNA photolyases (Li *et al*., 2004; Jenkins, 2009).

DNA damage can be significantly induced by higher levels of UV-B radiation and its self-repair mechanisms have also been investigated in many plant species, but information on the effects of exogenous hormones such as gibberellic acid on UV-B-induced DNA damage is very limited (Jenkins, 2009; Wang *et al*., 2016). Gibberellins (GAs) are a class of tetracyclic diterpenoid plant hormones generally involved in several physiological processes and biochemical metabolisms in plants (Hedden & Sponsel, 2015). Gibberellic acid (GA_3) is one of the most common plant growth regulators used in agriculture to control seed germination, leaf expansion, shoot elongation, organ differentiation, flowering and fruit maturation (Iqbal & Ashraf, 2013; Hedden & Sponsel, 2015; Pereira *et al*., 2017). To our knowledge, the effect of exogenously applied GA_3 on DNA damage and self-repair processes is still not reported in enhanced UV-B stressed wheat plants.

Therefore, the primary objective of our study is to determine if the application of exogenous GA_3 could improve seedling UV-B tolerance and UV-B induced-DNA damage phenomenon, and it would activate which specific processes to enhance DNA damage self-repair capacity in leaves of wheat seedlings under supplementary UV-B radiation.

Materials and Methods

Plant materials and growth conditions: *Triticum aestivum* (Jinmai 8) seeds were provided from Wheat Research Institute, Agricultural Sciences Academy, Shanxi Province, China. Uniform size and plump wheat seeds were selected, then surface sterilized for 30s with 0.1% $HgCl_2$, and rinsed three times by distilled water, then incubated in the clean Petri dishes (the diameter about 18.0cm) with wet gauze at a growth chamber under darkness for 3d at 28°C. At the same time, wheat seeds were also sown on wet gauze moistened with the solutions of 100, 150, or 300mg L^{-1} of exogenous GA_3, respectively.

Supplementary UV-B stress treatment was conducted after 3d pretreatment, just as seeds germination. After germination, the seeds were washed with distilled water, and transferred into clean Petri dishes, and incubated at plant growth chamber (10/14h day/night photoperiod, day/night temperature 24/18°C, 70% relative humidity). Supplementary UV-B light was provided by filter lamps (Model Qin brand, 30W, 297nm, Baoji Lamp Factory, Baoji City, China), and the filter lamps were hung above Petri dishes. The UV-B radiation intensity was determined with an UV radiometer (742 Optronics Instruments, Orlando, USA). The UV-B light radiation intensity was chosen as 10.08 KJ m^{-2} d^{-1} for 8h d^{-1} according to our previous experiments (Li *et al*., 2016; Li *et al*., 2017).

Growth parameter measurement: After supplementary UV-B stress for 3, 7, 10, 15, 20d, at least six individual wheat plants were harvested from different treatments for fresh weight (FW) determination. The leaf part of wheat was dried under 60°C conditions for over 48h, and the dry weight (DM) of wheat leaves was measured (Perveen & Nazir, 2018).

Determination of UV absorbance: UV-B-absorbing compound contents were determined according to the method of Wang *et al*., (2016). The fresh leaves of wheat seedlings were quickly frozen in liquid nitrogen (N_2), then homogenized in sodium phosphate buffer (50mM, pH 7.2, containing 10% (v/v) glycerol) with leaf tissue: buffer ratio at 1:15 (g: ml). The carotenoids and UV-B-absorbing compounds were completely extracted with 1.0% (v/v) HCl in 70% (v/v) methanol at 4°C conditions for 24h. The samples were centrifuged for 15min at 1,800×g. Absorbance of the extracts was determined at 330nm by using a UV-visible spectrophotometer (Hitachi 134 U-2900, Japan).

DNA extraction and damage assay: The CPDs and 6-4PPs levels of wheat leaves were quantified by enzyme linked immunosorbent assay described by Mori *et al*., (1991). The detailed procedure was conducted according to the manufacture instructions and the method of Li *et al*., (2000). The absorbance of reaction mixture was measured at 492nm with a UV-visible spectrophotometer (Hitachi 134U-2900, Japan).

DNA photolyase activities: The total DNA photolyase preparation and activity assay was performed according to the method of Hada *et al*., (2000). The DNA photolyase activity was measured with UV irradiated *Escherichia coli* DNA as substrates in the presence of egg protein extracts according to the method of van de Mortel *et al*., (1998).

Visualization of ROS accumulation: Histochemical localization of ROS including H_2O_2 and $O_2^{\cdot-}$ productions, was identified by the method according to our previous works (Li *et al*., 2016). The NBT or DAB staining intensity from different leaf tissues was calculated by the Image J software.

Results

Plant phenotypic response: Treatment of wheat seedlings with UV-B light alone resulted in significant alterations in plant phenotypic characters and growth parameters compared to controls. UV-B stressed-seedlings showed a green-yellow phenotype during the early growth stages (Fig. 1a). Growth of seedlings (including FW and DW values) also reduced by 70% and 40% under UV-B stress conditions compared to controls (Fig. 1b, c). Application of exogenous GA_3 effectively improved seedling phenotype and growth, in which 150mg L^{-1} of GA_3 seemed to be the most effective treatment.

The UV-B-absorbing compound concentrations: The concentration of UV-B-absorbing compound (UACs) was remarkably higher in wheat seedlings during the early stage of supplementary UV-B stress than those under normal conditions, and it would further increase in plants with different doses of GA_3 treatment (Fig. 2). Moreover, the UACs concentration was the highest in seedlings with 150mg L^{-1} of GA_3 treatment. In addition, UACs level decreased in seedlings after long-term UV-B irradiation (after more than 18d UV-B stress) compared with controls. Nevertheless, GA_3 treatment continuously maintained the higher level of UACs in seedling leaves.

DNA damage assay: The supplementary UV-B irradiation induced higher levels of CPD and 6-4PPs than the controls under normal growth conditions (Fig. 3a, b). Under increased UV-B stress, the use of exogenous GA_3 significantly decreased the concentrations of CPD and 6-4PPs in leaves of wheat seedlings. In accordance with our above results, the treatment with 150mg L^{-1} of GA_3 was the most effective for reducing DNA damage in wheat seedlings caused by UV-B radiation. DNA photolyases play an important role in photorepair mechanisms of CPD and 6-4PPs induced by UV-B stress, so in the subsequent experiments, we further determined DNA photolyase activity in leaves of wheat seedlings of different groups. According to the result of Fig. 3c, we also considered that exogenous GA_3 treatment could improve DNA photolyase activities, and the 150mg L^{-1} of GA_3 was the most efficient treatment.

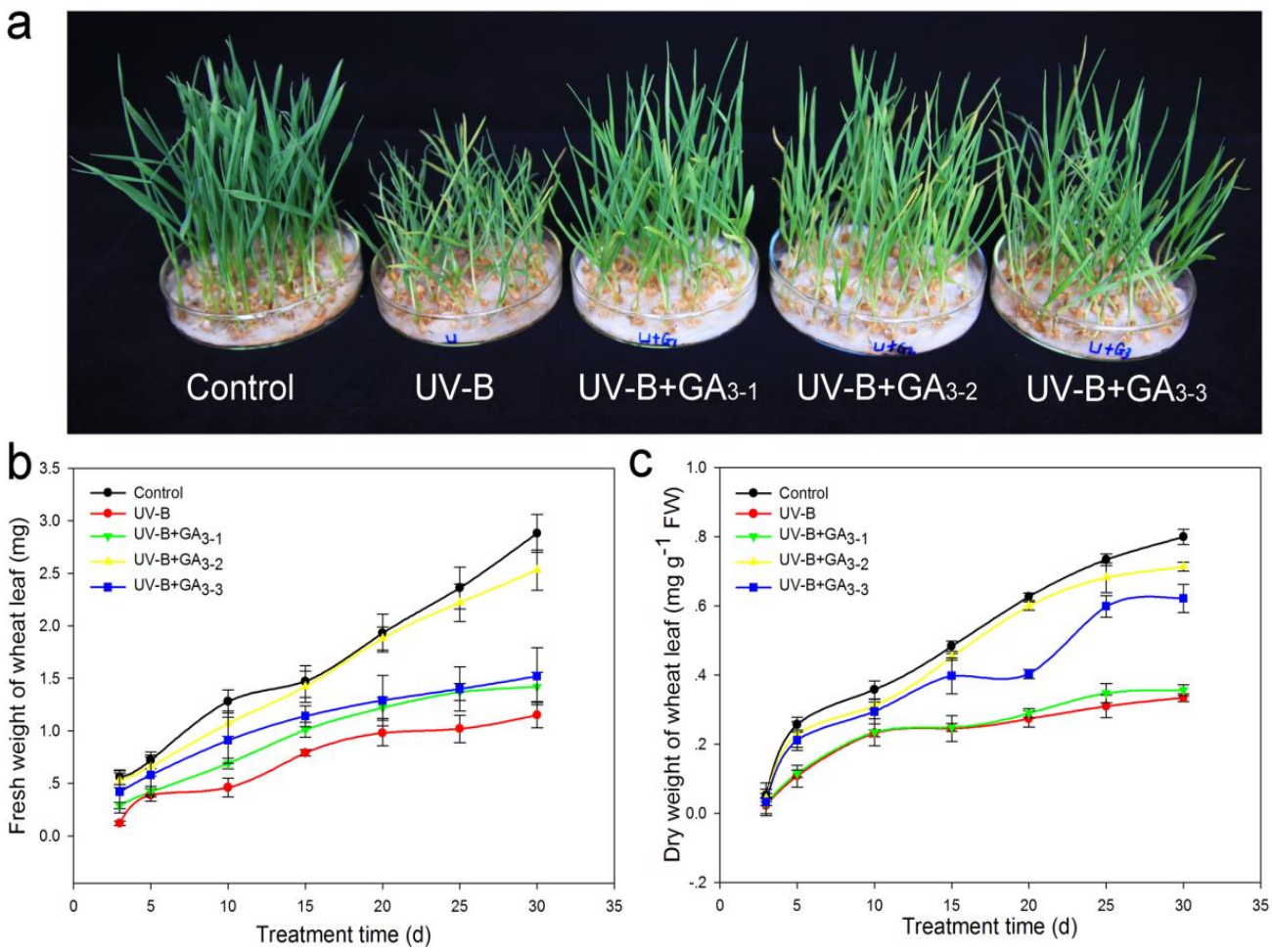

Fig. 1. Effects of exogenous GA_3 application on growth of wheat seedlings under supplementary UV-B stress. a. Phenotypical characterization of wheat seedlings under different growth conditions. b. Change of fresh weight (FW) in leaves of wheat seedlings. c. Change of dry weight (DW) in leaves of wheat seedlings. Control: Wheat seedlings under normal conditions; UV-B: The supplementary UV-B stress; UV-B+GA_{3-1}: The combination of supplementary UV-B stress and 100mg L^{-1} of GA_3 treatment; UV-B+GA_{3-2}: The combination of supplementary UV-B stress and 150mg L^{-1} of GA_3 treatment; UV-B+GA_{3-3}: The combination of supplementary UV-B stress and 300mg L^{-1} of GA_3 treatment. Data are averages ± SD (n=6).

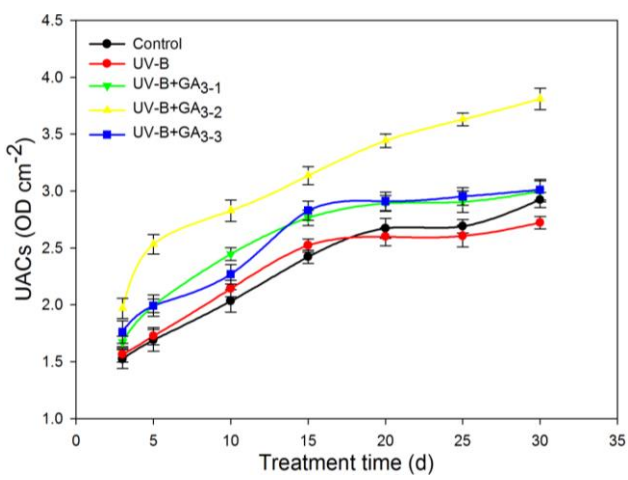

Fig. 2. Measurement of UV-B-absorbing compounds (UACs) in leaves of wheat seedlings. Control: Wheat seedlings under normal conditions; UV-B: The supplementary UV-B stress; UV-B+GA_{3-1}: The combination of supplementary UV-B stress and 100mg L^{-1} of GA_3 treatment; UV-B+GA_{3-2}: The combination of supplementary UV-B stress and 150mg L^{-1} of GA_3 treatment; UV-B+GA_{3-3}: The combination of supplementary UV-B stress and 300mg L^{-1} of GA_3 treatment. Data are averages ± SD (n=6).

ROS visualization: In the subsequent experiment, we examined ROS accumulation, such as H_2O_2 and $O_2^{\cdot-}$, in wheat leaves by histochemical stain technology according to our previous methods (Li *et al*., 2016). The results of our experiment found that UV-B stress increased ROS accumulation in plant leaves (Figs. 4-6), and the exogenous GA_3 application had not significantly altered supplementary UV-B stress-induced ROS accumulation. Therefore, we supposed that the protective effects of GA_3 on wheat seedlings under UV-B stress had no direct or close correlations with ROS production scavenging.

Discussion

UV-B light is one of the major environmental factors regulating growth, survival, development, reproduction, and geographic distribution of plants (Li *et al*., 2004; Jenkins, 2009). The present investigations have demonstrated that high levels of UV-B radiation are unfavorable for living organisms (Jenkins, 2009; Wang *et al*., 2016; Li *et al*., 2016). For example, the enhanced UV-B light will damage plant genomic DNA

and proteins, impedes DNA replication and transcription, and results to lethality or mutations (Kakani *et al*., 2003; Wang *et al*., 2016; Tripath *et al*., 2017). The increased UV-B radiation induced-DNA damages have been reported in many plants (Ciccia & Elledge, 2010). As we all known, UV light from sunlight can induce up to 10^5 DNA lesions (pyrimidine dimmers and 6-4PPs) per cell per day (Ciccia & Elledge, 2010). Additionally, chemical reagents and ROS productions induce the oxidation of DNA bases, and cause DNA double-strand (DSBs) breaks (Huang & Darzynkiewicz, 2006; Li *et al*., 2016). To protect against DNA damage, the specific repair mechanisms for various types of DNA lesion have evolved in plants. Mispaired bases in genomic DNA are replaced with correct bases via mismatch repair, and aberration of few DNA bases can be repaired through base excision repair due to the damaged base being excision. Additionally, those more complex DNA lesions can also be repaired by nucleotide excision repair pathway (Huang & Darzynkiewicz, 2006). DNA repair is usually carried out depending on a plethora of enzymatic activities that chemically modify DNA to repair DNA damage, such as photolyase, nuclease, helicase, polymerase, topoisomerase, recombinase, ligase, glycosylase, demethylase, kinase and phosphatase (Huang & Darzynkiewicz, 2006; Ciccia & Elledge, 2010).

The increased UV-B radiation commonly caused two types of DNA damage including CPDs and 6,4-PPs formation in living organisms (Fujimori *et al*., 2014). Although much is known about UV-B light-induced DNA damage repair pathways in plants, we know considerably less about the effects of exogenous GA_3 on the higher irradiance of UV-B light-induced DNA damage in wheat seedling leaves. In the present study, we have found that GA_3 treatment remarkably lessened the accumulation of DNA damage productions induced by the enhanced UV-B stress in wheat leaves. Furthermore, this ameliorated effect of GA_3 on DNA damage was concentration-dependent, with less damage at 150mg/L GA_3 than 100mg/L and 300mg/L (Fig. 3). This discovery of exogenous GA_3 application alleviating effects on UV-B induced DNA damage might be of important ecological implications. In natural environments, the solar UV-B radiation is often higher than regular level at high altitude, particularly in early spring when ozone layer depletion is more serious than other seasons in one year. Thus, identifying some effective physical or chemical methods to improve plant UV-B protection is very important (Li *et al*., 2016).

It was observed that efficiency of photorepair of both type of CPDs and 6-4PPs significantly increased with GA_3 treatment in UV-B stressed wheat leaves. DNA photolyase become more active in the presence of GA_3 conditions, indicating this lesion can be repaired mainly through photorepair mechanisms. In addition, UV-absorbing compound content also was higher in wheat leaves with GA_3 treatment than those without GA_3 treatment. Nevertheless, exogenous GA_3 application had not remarkably altered ROS accumulation in seedlings, so we supposed that cellular antioxidant systems had not play a role in the alleviating effects of GA_3 application on UV-B induced DNA damage.

The exogenous GA_3 treatment apparently facilitates DNA photorepair in plants, damage formation of CPDs and 6-4PPs are decreased, which could be trigger plant adaption mechanism to its environment. Difference of the repair capacity could contribute to changes in species distribution in areas affected by the enhanced UV-B radiation. Therefore, a much wider survey on the effects of some physical or chemical factors on UV-B-induced DNA damages and its self-repair is very essential.

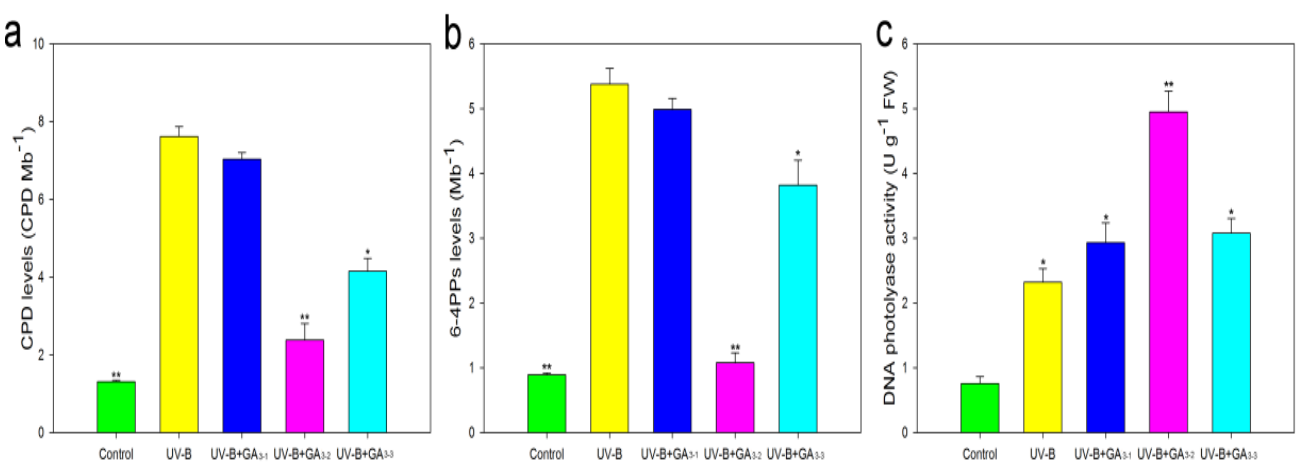

Fig. 3. DNA damage assay of wheat seedling under different growth conditions. a. CPD levels of wheat leaves. b. 6-4PPs levels of wheat leaves. c. DNA photolyase activities in wheat leaves. Control: Wheat seedlings under normal conditions; UV-B: The supplementary UV-B stress; UV-B+GA_{3-1}: The combination of supplementary UV-B stress and 100mg L^{-1} of GA_3 treatment; UV-B+GA_{3-2}: The combination of supplementary UV-B stress and 150mg L^{-1} of GA_3 treatment; UV-B+GA_{3-3}: The combination of supplementary UV-B stress and 300mg L^{-1} of GA_3 treatment. Data are averages ± SD (n=6). The * or ** followed with bars are significantly different at $p<0.05$ or $p<0.01$.

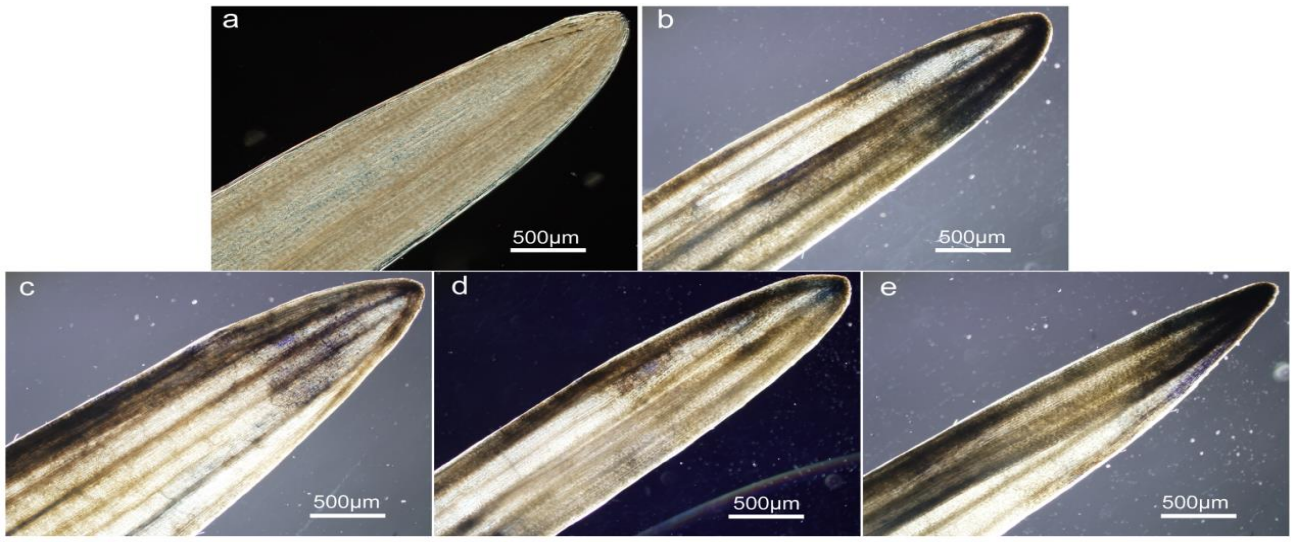

Fig. 4. Visualization of H_2O_2 accumulation in leaf tissue of wheat seedlings. a. Control. b. UV-B stress. c. The combination of 100mg L^{-1} of GA_3 application and UV-B stress. d. The combination of 150mg L^{-1} of GA_3 application and UV-B stress. e. The combination of 300mg L^{-1} of GA_3 application and UV-B stress. Bar=500μm.

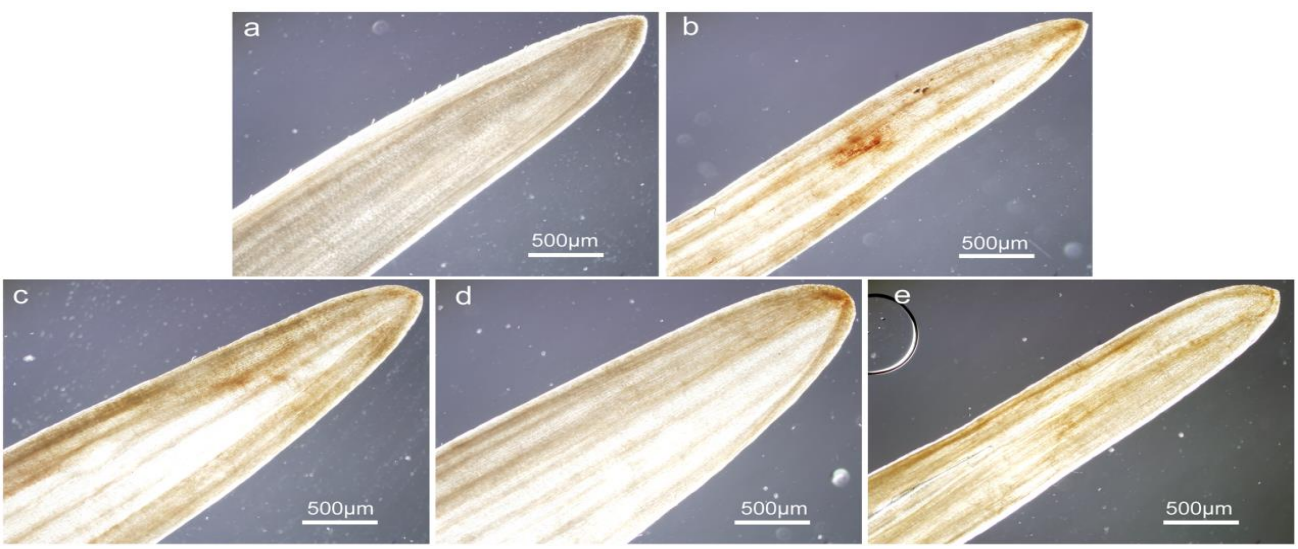

Fig. 5. Visualization of $O_2^{\cdot-}$ accumulation in leaf tissue of wheat seedlings. a. Control. b. UV-B stress. c. The combination of 100mg L^{-1} of GA_3 application and UV-B stress. d. The combination of 150mg L^{-1} of GA_3 application and UV-B stress. e. The combination of 300mg L^{-1} of GA_3 application and UV-B stress. Bar=500μm.

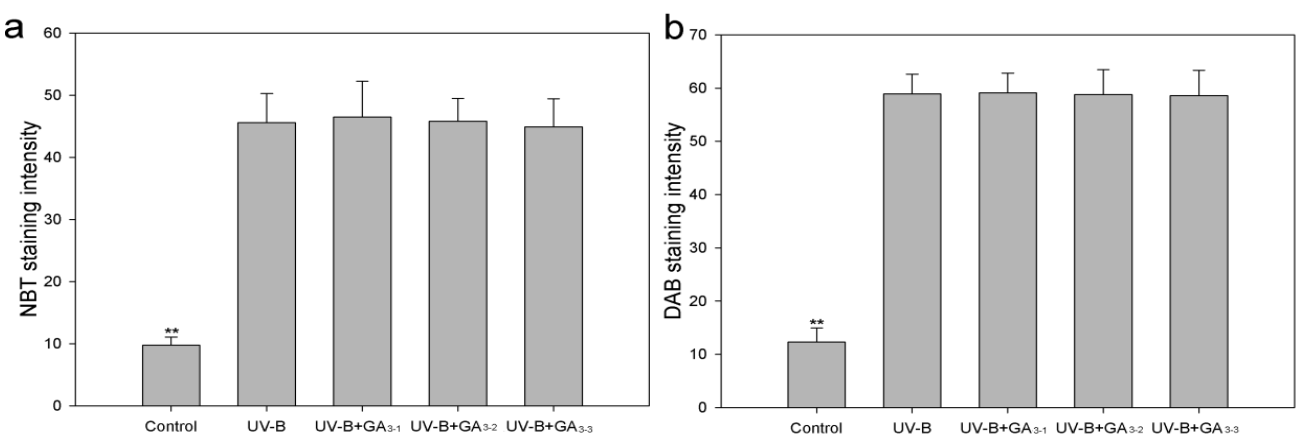

Fig. 6. ROS staining intensity analysis in leaves of wheat seedlings. Control: Wheat seedlings under normal conditions; UV-B: The supplementary UV-B stress; UV-B+GA_{3-1}: The combination of supplementary UV-B stress and 100mg L^{-1} of GA_3 treatment; UV-B+GA_{3-2}: The combination of supplementary UV-B stress and 150mg L^{-1} of GA_3 treatment; UV-B+GA_{3-3}: The combination of supplementary UV-B stress and 300mg L^{-1} of GA_3 treatment. Data are averages±SD (n=6). The * or ** followed with bars are significantly different at $p<0.05$ or $p<0.01$.

Conclusion

The excessive accumulation of cellular CPD and 6-4PPs have been monitored in wheat seedling leaves under continuous UV-B stress conditions, suggesting supplementary UV-B light radiation causes severe DNA damage for plant cells. Different concentrations of exogenous GA_3 application decreases CPD and 6-4PPs levels, and ameliorates UV-B induced DNA damage. Furthermore, 150mg/L GA_3 is the most efficiency treatment. We also have found that the biological effect of GA_3 is implemented mainly through activating DNA photorepair pathway and UV-absorbing compound biosynthesis, but has no obvious improvements for ROS metabolism. Therefore, we can concluded that the exogenous GA_3 partly alleviates the inhibition of increased UV-B stress on the growth and development of wheat seedlings and its phenotype due to lessening DNA damage and improving UACs biosynthesis.

Acknowledgements

We acknowledge the foundation of Higher School Science and Technology Innovation Project of Shanxi Province (Grant no. 20161106) and Cultivation Project of Youth Backbone Teacher of Shanxi Normal University.

References

Braun, C., Reef, R. and U.E. Siebeck. 2016. Ultraviolet absorbing compounds provide a rapid response mechanism for UV protection in some reef fish. *J. Photochem. Photobiol. B.*, 160: 400-407.

Britt, A.B. 2004. Repair of DNA damage induced by solar UV. *Photosyn. Res.*, 81: 105-112.

Brosché, N. and A. Strid. 2003. Molecular events of following perception of UV-B radiation by plants. *Physiol. Plant*, 117: 1-10.

Ciccia, A. and S.J. Elledge. 2010. The DNA damage response: making it safe to play with knives. *Mol. Cell*, 40: 179-204.

Fujimori, N., Suzuki, N., Nakajima, Y. and S. Suzuki. 2014. Plant DNA-damage repair/toleration 100 protein repairs UV-B-induced DNA damage. *DNA Repair.*, 21: 171-176.

Hada, M., K. Hino, G. Buchholz, J. Goss, E. Wellmann and M. Shin. 2000. Assay of DNA photolyase activity in Spinach leaves in relation to cell compartmentation-Evidence for lack of DNA photolyase in chloroplasts. *Biosci. Biotech. Biochem.*, 64: 1288-1291.

Hedden, P. and V. Sponsel. 2015. A century of gibberellins research. *J. Plant Growth Regul.*, 34: 740-760.

Huang, X. and Z. Darzynkiewicz. 2006. Cytometric assessment of histone H2AX phosphorylation: a reporter of DNA damage. *Methods Mol. Biol.*, 314: 73-80.

Interdonato, R., M. Rosa, C.B. Nieva, J.A. González, M. Hilal and F.E. Prado. 2011. Effects of low UV-B doses on the accumulation of UV-B absorbing compounds and total phenolics and carbohydrate metabolism in the peel of harvested lemons. *Environ. Exp. Bot.*, 69: 204-211.

Iqbal, M. and M. Ashraf. 2013. Gibberellic acid mediated induction of salt tolerance in wheat plants: growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. *Environ. Exp. Bot.*, 86: 76-85.

Jenkins, G.I. 2009. Signal transduction in responses to UV-B radiation. *Annu. Rev. Plant Biol.*, 60: 407-431.

Kakani, V.G., K.R. Reddy, D. Zhao and K. Sailaja. Field crop responses to ultraviolet-B radiation: a review. *Agr. Forest Meteorol.*, 120: 191-218.

Liakopoulos, G., S. Stavrianakou and G. Karabourniotis. 2006. Trichome layers versus dehaired lamina of Olea europaea leaves: differences in flavonoid distribution, UV-absorbing capacity, and wax yield. *Environ. Exp. Bot.*, 55: 294-304.

Li, Y.F., L.M. Gao and R. Han. 2016. A combination of He-Ne laser irradiation and exogenous NO application efficiently protect wheat seedling from oxidative stress caused by elevated UV-B stress. *Environ. Sci. Pollut. Res.*, 23: 23675-23682.

Li, Y.F., L.M. Gao and R. Han. 2016. Endogenous nitric oxide mediates He-Ne laser-induced adaptive responses in salt-stressed tall fescue leaves. *Biosci. Biotech. Biochem.*, 80: 1887-1897.

Li Y.F., L.M. Gao and R. Han. 2017. He-Ne laser illumination ameliorates photochemical impairment in Ultraviolet-B stressed-wheat seedlings via detoxifying ROS cytotoxicity. *Russ. J. Plant Physiol*., 5: 766-775.

Li, S., Y. Wang and X. Wang. 2000. Cyclobutane pyrimidine dimer accumulation in relation to UV-B sensivity in rice cultivars. *Acta Botanica. Sinica*., 42: 576-581.

Li, S.S., Y. Wang and L.O. Björn. 2004. Effects of temperature on UV-B-induced DNA damage and photorepair in *Arabidopsis thaliana*. *J. Environ. Sci.*, 16: 173-176.

Monforte, L., R. Tomás-Las-Heras, M. Del-Castillo-Alonso, J. Martínez-Abaigar and E. Núñez-Olivera. 2015. Spatial variability of ultraviolet-absorbing compounds in an aquatic liverwort and their usefulness as biomarkers of current and past UV radiation: A case study in the Atlantic Mediterranean transition. *Sci. Total Environ.*, 518-519: 248-257.

Monforte, L., E. Núñez-Olivera and J. Martínez-Abaigar. 2015. UV radiation biomonitoring using cell compartmentation of UV-absorbing compounds in herbarium samples of a liverwort. *Ecol. Indic.*, 52: 48-56.

Mori, T., M. Nakane and T. Hattori. 1991. Simultaneous establishment of monoclonal antibodies specific for either cyclobutane pyrimidine dimer or (6,4) photoproduct from the same mouse immunized with ultraviolet irradiated DNA. *Photochem. Photobiol.*, 54: 225-232.

Pereira, A.E.S., P.M. Silva, J.L. Oliveira, H.C. Oliveira and L.F. Fraceto. 2017. Chitosan nanoparticles as carrier systems for the plant growth hormone gibberellic acid. Colloid Surface B., 150: 141-152.

Perveen, S. and M. Nazir. 2018. Proline treatment induces salt stress tolerance in maize (*Zea mays* L. CV. Safaid Afgoi). *Pak. J. Bot.*, 50: 1265-1271.

Rozema, J., J. van de Staaij, L.O. Björn and M. Caldwell. 1997. UV-B as an environmental factor in plant life: stress and regulation. *Trends in Ecol. Evol.*, 12: 22-28.

Sancar, A., L.A. Lindsey-Boltz, K. Unsal-Kacmaz and S. Linn. 2004. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu. Rev. Biochem.*, 73: 39-85.

Tripath, D.K., S. Singh, V.P. Singh, S.M. Prasad, N.K. Dubey and D.K. Chauhan. 2017. Silicon nanoparticles more effectively alleviated UV-B stress than silicon in wheat (*Triticum aestivum*) seedlings. *Plant Physiol. Biochem.*, 110: 70-81.

van de Mortel, T., W. Buttemer, H. Hoffman, J. Hays and A. Blaustein. 1998. A comparison of photolyase activity in three australian tree frogs. *Oecologia.*, 115: 366-369.

Wang, Q.W., S. Nagano, H. Ozaki, S.I. Morinaga, J. Hidema and K. Hikosaka. 2016. Functional differentiation in UV-B induced DNA damage and growth inhibition between highland and lowland ecotypes of two *Arabidopsis* species. *Environ. Exp. Bot.*, 131: 110-119.

(Received for publication 8 November 2017)