ISSN 1021-4437, Russian Journal of Plant Physiology, 2017, Vol. 64, No. 5, pp. 766–775. © Pleiades Publishing, Ltd., 2017.

RESEARCH PAPERS

He–Ne Laser Illumination Ameliorates Photochemical Impairment in Ultraviolet-B Stressed-Wheat Seedlings Via Detoxifying ROS Cytotoxicity[1]

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Received December 4, 2015

Abstract—The protective effect and physiochemical mechanism of He-Ne laser illumination on photochemical impairment were evaluated by investigating chlorophyll fluorescence characteristics, photochemical activities of two photosystems, reactive oxygen species (ROS) levels and antioxidant enzyme activities in UV-B stressed-wheat (*Triticum aestivum* L.) seedlings. The results showed that enhanced UV-B stress significantly inhibited plant growth, reduced photosynthetic pigment content and antioxidant enzyme activities, while increased intracellular ROS levels. Meanwhile, UV-B stress also altered chlorophyll fluorescence characteristics and photochemical activities of seedlings. However, He-Ne laser illumination markedly improved photochemical activities and photosynthetic efficiency of two photosystems through detoxifying excessive ROS productions. Illumination with white fluorescent lamps (W), red light (R), or red light, then far-red light (R + FR) had not alleviated the inhibitory effect of UV-B stress on plant growth, suggesting that He-Ne laser illumination might be responsible for UV-B-stressed seedlings due to its regulation for intracellular ROS levels and plant oxidant/antioxidant balance. Furthermore, the laser alone also showed a positive impact on photochemical activities of photosystem I and photosystem II in plants.

Keywords: Triticum aestivum, photochemical impairment, enhanced ultraviolet-B stress, He–Ne laser, wheat seedlings, chlorophyll fluorescence

DOI: 10.1134/S1021443717050041

INTRODUCTION

Recently, the global atmospheric pollution and ecosystem destruction are becoming more and more serious [1]. The stratospheric ozone (O_3) has been constantly depleted due to the various anthropogenic activities, such as the utility and release of chlorofluorocarbons, nitrogen oxides and methyl bromide, which would result in ultraviolet light enhancement that reaches the earth surface [1, 2]. Cellular components including nucleic acids, proteins, lipids and various physiologically active substances like vitamin and quinines, can absorb ultraviolet light (we refer to the 280 ~ 320 nm wavelengths as being in the ultraviolet-B region of the spectrum) [3]. The lower intensity of UV-B light usually induces the synthesis of various compounds, consisting of low molecular weight antioxidants, antioxidant enzymes, and chaperone proteins that can activate the protective systems in plants [3, 4]. However, enhanced UV light radiation, especially UV-B, has potentially threatened plant growth and development [3–6].

For higher plants, the photosynthetic apparatus with potential sites of superoxide production is one of the most important targets for UV light (Fig. 1) [7–11]. Although plants can protect photosynthetic apparatus by a series brand spectrum of defense mechanisms, photosynthetic apparatus is still seriously injured under the higher irradiance of UV-B stress. There are evidences that He–Ne and CO_2 laser can protect plants from physiological damages under various unfavorable environments [11–16]. However, the actual protective effect and physiochemical mechanism of He–Ne laser on photosynthetic damages

[1] The article is published in the original.
Supplementary materials are available for this article at DOI 10.1134/S1021443717050041 and are accessible for authorized users.

Abbreviations: APX—ascorbate peroxidase; CAT—catalase; POD—peroxidase; R—red light; R + FR—red light + far-red light; SOD—superoxide dismutase; W—white fluorescent.

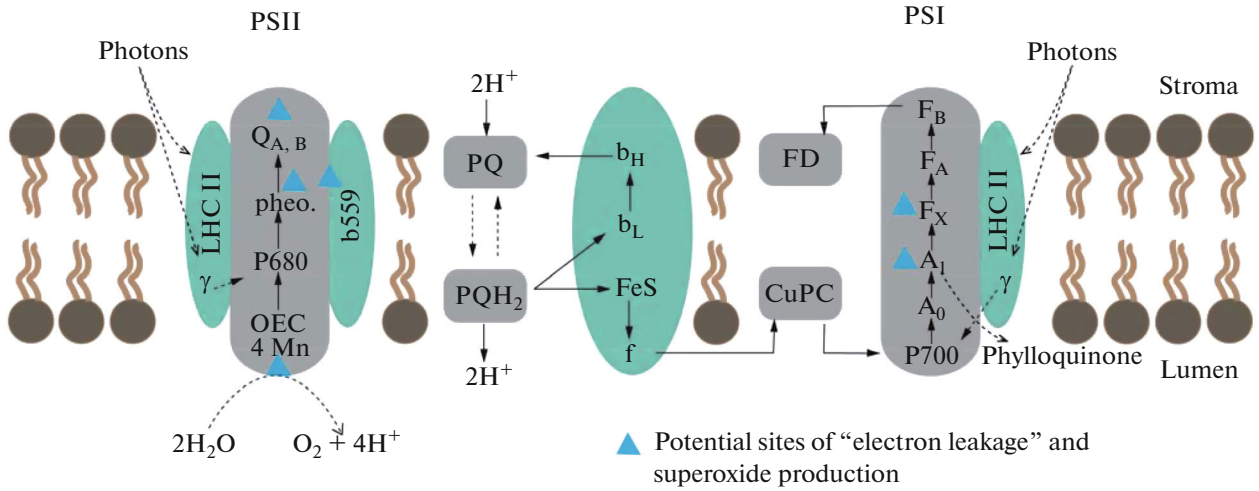

Fig. 1. Hypothetical potential sites of ROS generation in two photosystems under enhanced UV-B stress.

caused by enhanced UV-B stress are still largely unknown. To gain better insight into protective mechanism of He–Ne laser against UV-B stress, the photochemical activities of PSI and PSII were both examined in our experiments, despite PSII is more sensitive to UV-B light than PSI.

The induction of chlorophyll (Chl) fluorescence is an effective and sensitive measure of photosynthetic energy conversion. Previous studies showed that Chl fluorescence had been applied extensively for rapid assessment of energy conversion efficiencies in PSI and PSII [17]. The effects of environmental factors on photosynthesis had been investigated in many plants, including perennial fruiting plants, cereal crops, *Peganum harmala* and tree species by the use of Chl fluorescence parameters as a selection tool [17]. Therefore, Chl fluorescence was a natural biomaker in plants for routinely monitoring of environmental stress. Some researchers also assumed that there was a link between Chl fluorescence characteristics of PSI and PSII, and the laser-induced adaptive responses to a variety of natural stressors. However, to our knowledge, there is no report by the use of Chl fluorescence as a biomarker for the protective effect of He-Ne laser on enhanced UV-B-induced photochemical impairment. These problems need further investigation in our work presented here. Plant antioxidant system, consisting of a variety of antioxidant compounds and antioxidant enzymes, plays an important role in the formation of plant resistance to different stress factors and the development of stress-protective mechanisms in plants [18, 19]. Thus, we have assumed that plant antioxidant system is also probably involved in the protective effects of He–Ne laser on photochemical impairments in UV-B-stressed plants. Therefore, we subsequently also examined antioxidant enzyme activities and ROS levels.

Wheat is one of the most important cereal crops and widely cultivated in many countries all over the world, and it serves three main purposes: food supply for human consumption, feed for livestock and poultry, and raw materials for agro-based industry. It is very necessary to maintain the higher yield of wheat, especially under extreme field conditions. Therefore, improved the tolerance of wheat to a variety of unfavorable environment conditions might contribute to substantially improving the yield of crops in many regions.

In the present study, the photochemical activity of photosystem I and photosystem II in leaves of wheat seedlings subjected to a combination of enhanced UV-B stress and He–Ne laser were evaluated by measuring photosynthetic pigment contents, Chl fluorescence parameters, ROS levels and plant antioxidant system. The main objective of our paper is to explore the protective effect and biochemical mechanism of He–Ne laser irradiation on UV-B induced photochemical impairments.

MATERIALS AND METHODS

Material culture and treatment. Winter wheat (*Triticum aestivum* L. cv. Jinmai 8) seeds were obtained from Wheat Research Institute, Shanxi Agricultural Sciences Academy, China. Uniform size and plump seeds were selected and surface sterilized with 0.1% $HgCl_2$ for 30 s, rinsed three times with distilled water, then incubated in Petri dishes (diameter 18 cm) with wet filter paper at a growth chamber for 3 d at 28°C. Stress treatments were carried out after 3 d of pre-culture, just as seeds germination. The UV-B radiation (B) was generated by a filter lamp (Qin brand, Baoji Lamp Factory, China, 30 W, 297 nm), and the lamps were hung above Petri dishes. The radiation intensity of lamps was determined with an Optronics spectroradiometer (Model 742 Optronics Laboratories, United States). The UV-B radiation intensity was 2.8 W m^{-2} for 8 h d^{-1} according to the

method of Yang et al. [6]. The wavelength of He-Ne laser (L) generator (MSHN-A-B450MM, Nanjing Laser Instrument Factory, China) was 632.8 nm, initial beam diameter 2 mm and final diameter 100 mm via amplifier, and the flux rate 5.43 mW mm^{-2} for 4 min d^{-1} at 25°C for 7 d [6]. At the same time, control plants (CK) without any stress treatment were grown at 25°C, 70% relative humidity, photoperiod 16/8 h light/dark cycle. Light was provided by 100 μmol/(m^2 s) of fluorescent lamp.

To further demonstrate the biological effects of He-Ne laser illumination on UV-B stressed-wheat seedlings, the white fluorescent lamps (W, photosynthetic photo flux density (PPFD), 100 μmol/(m^2 s), 10 min), red light with the same wavelength (R, λ_R = 633 nm, 10 min), a combination of red light and far-red light (red light irradiation, then far-red light, R + FR, λ_{RL} = 633 nm, $\lambda_{FR,max}72$ = 7 nm, half-width 40 nm, 10 min), obtained with the help of LEDs, are also applied in plants for 7 d prior to enhanced UV-B stress. The photosynthetic photo flux density was 100 μmol/(m^2 s d) on the surface of leaves with different light sources, including white fluorescent, 633 nm red light alone, and red light+ far-red, according to Kreslavski et al. [3]. Such low irradiance was used to check out the involvement of phytochrome-controlled reversible low-fluency responses. Laser and all fluorescence lamps illumination were carried out at a.m. 6:00-6:30 in darkness to prevent the influence of stray light. After UV-B stress 10 day, the 10-day-old of wheat seedlings were harvested for further analysis.

Phenotypic alterations of wheat seedlings. In present study, we observed the phenotypic alterations of wheat seedlings under different conditions. The effects of combined treatments with He–Ne laser illumination and enhanced UV-B stress on morphological characteristics of wheat seedlings were also evaluated by the estimation of agronomic characters, such as plant height, root length and biomass.

Photosynthetic pigment content measurement. Fresh leaves of wheat seedlings were collected for further measurement. Photosynthetic pigments were extracted by the method of Qu et al. [20]. Chlorophyll (a/b) and carotenoid content were measured spectrophotometrically (Hitachi U-2900, Japan) at 646 nm, 663 nm and 470 nm, respectively, and calculated by the method of Porra et al. [21].

Chlorophyll fluorescence measurement. Chl fluorescence parameters of PSII were recorded with a portable pulse-amplitude modulation fluorometer (PAM-2100, Heinz Walz, Germany) at 25°C according to the manufacturer's instructions. Leaves were dark-adapted in the sample chamber for 30 min prior to all measurements. The maximum fluorescence yield (Fm) was measured by application of 0.8 s pulse of light of high intensity (PPFD > 4000 μmol/(m^2 s)). The maximum quantum efficiency of PSII primary photochemistry (Fv/Fm) was estimated as

$$(Fm - Fo)/Fm.$$

PSI parameters and ETR of two photosystems were recorded with a dual wavelength pulse-amplitude modulated fluorescence monitoring system (Dual-PAM-100, Heinz Walz). The energy dissipation parameters in PSI and PSII were calculated according to the manufacturer's instructions.

The non-photochemical quantum yield accepted side of PSI was estimated as

$$Y(NA) = (Pm - Pm')/Pm,$$

where Pm and Pm' are the maximum change of the P700 signal in the dark-adapted and light-adapted situation, respectively.

The non-photochemical quantum yield donor side of PSI was calculated as

$$Y(ND) = 1 - P700red,$$

where P700red was the redox change of P700.

The values of ETR were obtained by the formula:

$$ETR = \Delta F/Fm' \times PAR \times 0.5 \times 0.84.$$

All parameters were conducted with the uppermost two leaves of single wheat seedlings.

Enzymatic activities measurements of SOD, POD, APX and CAT. Superoxide dismutase (SOD) activity was measured according to Rao and Sresty method [22]. Peroxidase (POD) and catalase (CAT) activity were determined as described by the method of Zhang and Kirham [23]. Measurements of ascorbate peroxidase (APX) were carried out according to Nakano and Asada method [24].

Visualization of H_2O_2 and O_2^-. Fresh leaves of wheat seedlings were collected for ROS levels assay. Intracellular H_2O_2 and O_2^- levels in seedling leaves were visualized by the method of Cembrowska-Lech et al. [25].

Statistical analysis. Values from five replicates were presented as mean ± standard deviation (SD) and evaluated with an analysis of variance (ANOVA). Data differences were considered significant at $P < 0.05$ according to Duncan's multiple range test.

RESULTS

Phenotypic Characteristics of Wheat Seedlings

According to Fig. 2, we found that enhanced UV-B stressed-plants showed a green-yellow phenotype before two-leaf stage of wheat (B). After two-leaf stage, yellow leaves gradually turned green, and eventually approached normal color. After He–Ne laser illumination, all leaves showed normal green color (BL). Moreover, He-Ne laser treatment alone (L) also improved plant phenotype compared with controls.

The effects of combined treatments with He–Ne laser and UV-B stress on morphological characteristics of wheat seedlings were also evaluated by the esti-

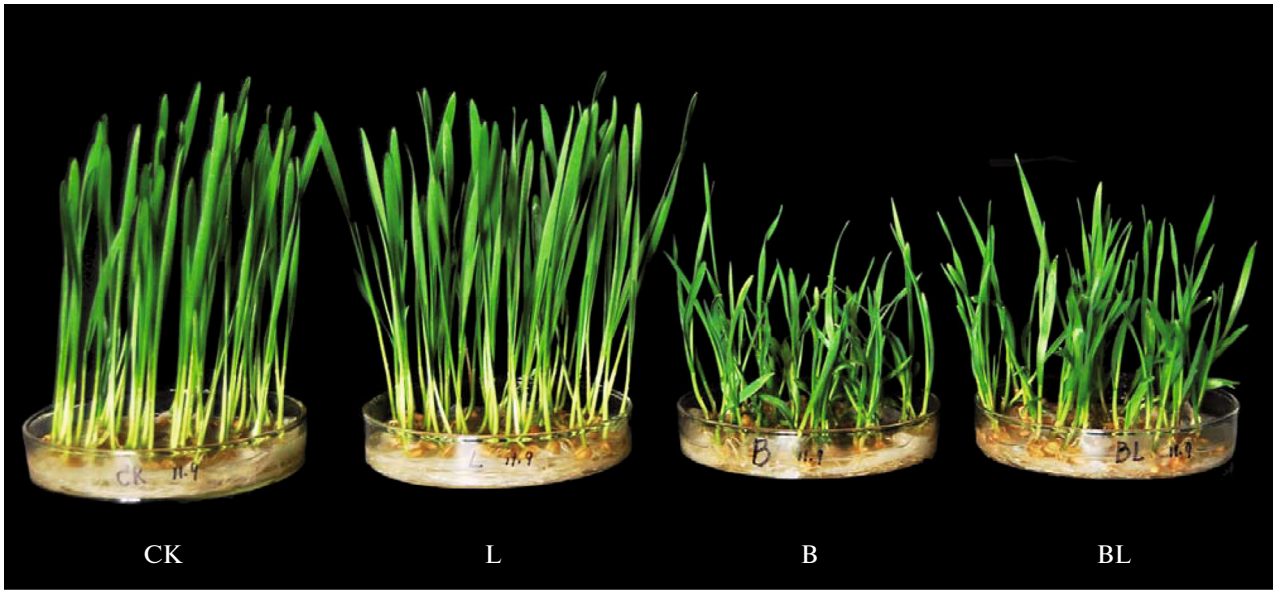

Fig. 2. The phenotype of wheat seedlings under different growth conditions. CK—control wheat seedlings without any stress or other treatment; L—plants radiated by He-Ne laser for 4 min d^{-1} (without UV-B radiation); B—plants stressed with enhanced UV-B for 8 h d^{-1}; BL—plants with combined treatment of enhanced UV-B stress for 8 h d^{-1} and He–Ne laser for 4 min d^{-1}.

mation of agronomic characters. As shown in Table 1, plant height, fresh weight, dry weight and root length significantly decreased in seedlings under enhanced UV-B stress, compared with controls. Plants illuminated with He–Ne laser and UV-B stress, however, were less affected.

Illumination with red light alone (BR), red light + far-red (BR+FR), white fluorescent lamps (BW) had no obvious improved effects on phenotype and growth of UV-B stressed-seedlings, which was indicated by plant height, fresh weight, soluble protein contents and the soluble sugar contents (Supplementary materials, Fig. 1a–d). Thus, in the subsequent experiment, we selected He–Ne laser illumination to treat plants materials under UV-B stress.

Photosynthetic Pigment Content

Photosynthetic pigment (Chl a/b, carotenoid) contents of wheat seedlings under UV-B stress remarkably decreased compared with controls (Fig. 3). Nevertheless, photosynthetic pigment contents of plants exposed to combined treatments were significantly higher than UV-B stressed-plants, still lower than controls. He-Ne laser treatment alone also significantly increased pigment contents.

Quantum Yield of Photosystem II

Table 2 showed that UV-B stress greatly reduced the value of Fv/Fm of seedlings compared with control plants. Moreover, the quantum yield of PSII (YII) also remarkably decreased under enhanced UV-B stress. However, He–Ne laser illumination effectively attenuated the reduction of Fv/Fm and Y(II) of wheat seedlings PSII under UV-B stress.

The values of the quantum yield of PSII energy dissipation parameters, including Y(NPQ) and Y(NO), in wheat seedlings were significantly increased when under UV-B stress. The values of energy dissipation parameters were decreased by He–Ne laser, which was similar with controls. Besides, He–Ne laser alone had a positive impact on the values of Y(NPQ) and Y(NO) in PSII.

Table 1. Effect of different treatments on growth parameters of 10-day-old of wheat seedlings

Treatment	CK	L	B	BL
Plant height, cm	9.86 ± 0.66a	13.35 ± 0.58b	5.57 ± 0.49c	8.98 ± 0.75a
Fresh weight, g	0.56 ± 0.034a	0.84 ± 0.033b	0.21 ± 0.056c	0.51 ± 0.032a
Dry weight, g	0.042 ± 0.002a	0.061 ± 0.003b	0.018 ± 0.003c	0.039 ± 0.007a
Root length, cm	6.42 ± 0.63a	8.95 ± 0.73b	3.24 ± 0.54c	5.93 ± 0.81a

Values are means ± SD calculated from 5 replicated measurements, $n = 5$, and values in the same row followed by different letters are significantly different ($P < 0.05$). CK—wheat seedlings without any stress or other treatment; L—plants radiated by He–Ne laser for 4 min d^{-1}(without UV-B radiation); B—plants stressed with enhanced UV-B for 8 h d^{-1}; BL—plants with combined treatment of enhanced UV-B stress for 8 h d^{-1} and He–Ne laser for 4 min d^{-1}.

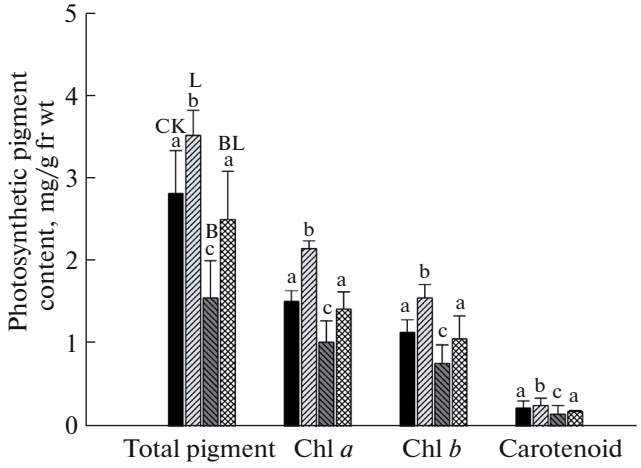

Fig. 3. Effects of different treatments on photosynthetic pigment content of wheat seedlings. CK—control wheat seedlings without any stress or other treatment; L—plants radiated by He–Ne laser for 4 min d^{-1} (without UV-B radiation); B—plants stressed with enhanced UV-B for 8 h d^{-1}; BL—plants with combined treatment of enhanced UV-B stress for 8 h d^{-1} and He–Ne laser for 4 min d^{-1}. Treatments with different letters indicate significantly different at $P < 0.05$. Values represent means ± SD calculated from 5 replicated measurements for each treatment, $n = 5$.

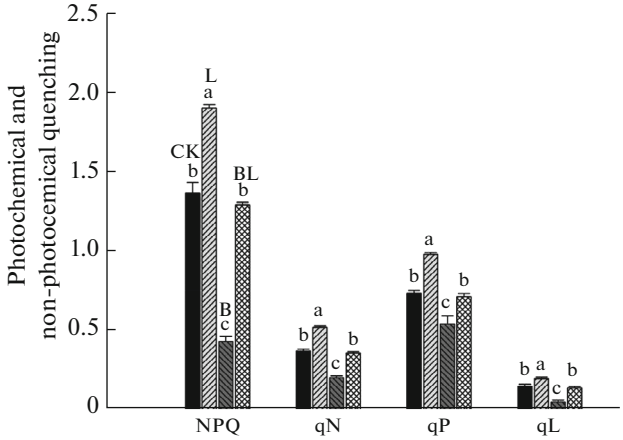

Fig. 4. The photochemical and non-photochemical quenching in leaves of wheat seedlings under different treatment conditions. qP, qL—photochemical quenching coefficient; NPQ, qN—non photochemical quenching coefficient. CK—control wheat seedlings without any stress or other treatment; L—plants radiated by He–Ne laser for 4 min d^{-1} (without UV-B radiation); B—plants stressed with enhanced UV-B for 8 h d^{-1}; BL—plants with combined treatment of enhanced UV-B stress for 8 h d^{-1} and He–Ne laser for 4 min d^{-1}. Treatments with different letters indicate significantly different at $P < 0.05$. Values represent means ± SD calculated from 5 replicated measurements for each treatment, $n = 5$.

Quantum Yield of Photosystem I

Under UV-B stress, the photochemical efficiency (YI) and non-photochemical quantum yield accepted side Y(NA) in PSI of wheat seedlings were significantly decreased, but non-photochemical quantum yield donor side Y(ND) in PSI was significantly increased compared with controls (Table 3). However, He-Ne laser illumination remarkably attenuated the reductions of (YI) and Y(NA), as well as the increases of Y(ND). In addition, He-Ne laser alone also had a positive effect on PSI quantum yield.

Photochemical and Non-Photochemical Quenching of Photosystem II

As shown in Fig. 4, the photochemical quenching (qP and qL) and non-photochemical quenching (qN and NPQ) of wheat seedlings under UV-B stress significantly decreased compared to controls. The quenching values of wheat seedlings under a combination of the laser and UV-B stress were higher than those of UV-B-stressed plants, but still lower than controls. He–Ne laser alone had a positive effect on these parameters.

Electron Transport Rate of Photosystem I and Photosystem II

To further investigate the influence of enhanced UV-B stress and He-Ne laser illumination on electron transport of two photosystems in wheat seedlings, electron transport rates of PSI and PSII were measured and analyzed, respectively (Fig. 5). The electron transport rate (ETRI and ETRII) in leaves of wheat seedlings under UV-B stress both decreased compared to controls. However, the ETRI and ETRII of seed-

Table 2. The quantum yield of photosystem II of 10-day-old of wheat seedlings from four groups

Treatment	CK	L	B	BL
Fv/Fm	0.862 ± 0.023a	0.888 ± 0.039b	0.538 ± 0.036c	0.727 ± 0.032d
Y(II)	0.245 ± 0.025c	0.214 ± 0.020d	0.365 ± 0.009a	0.290 ± 0.023b
Y(NO)	0.352 ± 0.017a	0.372 ± 0.021b	0.409 ± 0.014c	0.401 ± 0.021d
Y(NPQ)	0.302 ± 0.016a	0.279 ± 0.025b	0.321 ± 0.015ac	0.337 ± 0.022c

Values are means ± SD calculated from 5 replicated measurements, $n = 5$, and values in the same row followed by different letters are significantly different ($P < 0.05$). CK—wheat seedlings without any stress or other treatment; L—plants radiated by He-Ne laser for 4 min d^{-1}(without UV-B radiation); B—plants stressed with enhanced UV-B for 8 h d^{-1}; BL—plants with combined treatment of enhanced UV-B stress for 8 h d^{-1} and He–Ne laser for 4 min d^{-1}.

Table 3. The quantum yield of 10-day-old of wheat seedlings photosystem I in different groups

Treatment	CK	L	B	BL
Y(I)	0.423 ± 0.026a	0.450 ± 0.014a	0.280 ± 0.020b	0.351 ± 0.022c
Y(ND)	0.339 ± 0.012a	0.372 ± 0.026a	0.539 ± 0.026b	0.429 ± 0.011c
Y(NA)	0.472 ± 0.026a	0.498 ± 0.021a	0.142 ± 0.012b	0.334 ± 0.015c

Values are means ± SD calculated from 5 replicated measurements, $n = 5$, and values in the same row followed by different letters are significantly different ($P < 0.05$). CK—wheat seedlings without any stress or other treatment; L—plants radiated by He–Ne laser for 4 min d^{-1}(without UV-B radiation); B—plants stressed with enhanced UV-B for 8 h d^{-1}; BL—plants with combined treatment of enhanced UV-B stress for 8 h d^{-1} and He–Ne laser for 4 min d^{-1}.

lings under the combined treatments were both significantly enhanced.

Antioxidant Enzyme Activities and ROS Parameters

Our results also showed that enhanced UV-B stress significantly decreased enzymatic activities of SOD, POD, APX and CAT, whereas increased ROS levels in plants (Fig. 6). He–Ne laser illumination ameliorated the inhibitory effects of UV-B stress on plant antioxidant system and physiological parameters. Moreover, He–Ne laser alone also had a favorable impact on the values of these biochemical parameters and physiological parameters compared with controls.

DISCUSSION

Plants grown under field conditions are often subjected to a variety of different environmental stressors, such as high salinity, drought, extreme temperature, UV-B stress, heavy metals and nanoparticles [26–29]. These environmental stressors cause significant decreases in plant growth and development due to the disruption of biochemical metabolism and physiological processes, especially photosynthesis [20]. The high amount of UV-B radiation has harmful effects on the aerial parts of plants, because underground parts, such as roots, are usually protected from UV-B radiation [6]. Consequently, the retardation of plant growth and development is most likely attributed to the reduction of photosynthetic efficiency caused by UV-B radiation. When plants were exposed to enhanced UV-B radiation, photosynthetic pigment biosynthesis capacity was decreased, as indicated by the lower photosynthetic pigment contents (Fig. 3). In agreement with our results, Yang et al. [6] and Chen et al. [12] also reported that decreased chlorophyll contents have been associated with enhanced UV-B stress. He-Ne laser illumination showed the positive effects on photosynthetic pigment biosynthesis in UV-B-stressed seedlings. Moreover, He–Ne laser alone also enhanced photosynthetic pigment biosynthesis compared to controls. Therefore, we concluded that He–Ne laser improved photosynthetic efficiency through enhancement photosynthetic pigment biosynthesis capacity.

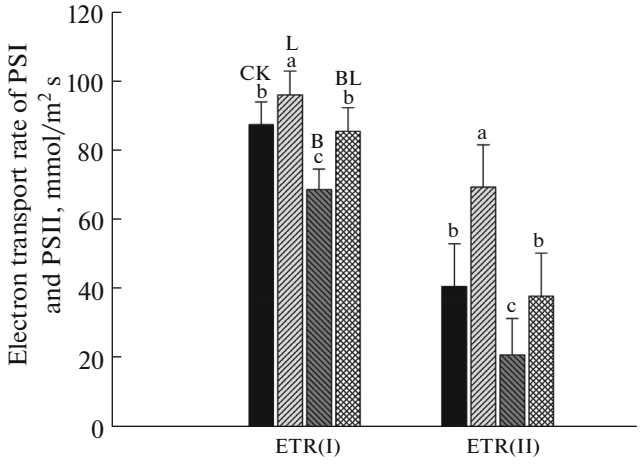

Fig. 5. Electron transport rates in leaves of wheat seedlings. CK—control wheat seedlings without any stress or other treatment; L—plants radiated by He–Ne laser for 4 min d^{-1} (without UV-B radiation); B—plants stressed with enhanced UV-B for 8 h d^{-1}; BL—plants with combined treatment of enhanced UV-B stress for 8 h d^{-1} and He–Ne laser for 4 min d^{-1}. Treatments with different letters indicate significantly different at $P < 0.05$. Values represent means ± SD calculated from 5 replicated measurements for each treatment, $n = 5$.

Chl fluorescence is commonly employed in the evaluation of energy conversion efficiency of photosynthesis, and is also considered as a biomarker of environmental stressors-induced photochemical impairments. To gain better insight into protective mechanism of He–Ne laser on photochemical impairment against UV-B stress, Chl fluorescence and light reaction kinetics in PSI and PSII was measured, respectively. UV-B stress caused significant decreases in quantum yield, photochemical efficiency, photochemical quenching, non-photochemical quenching and electron transport rate, and significant increases in parameters of quantum yield of energy dissipation of two photosystems. However, He–Ne laser illumination significantly reversed the values of Chl fluorescence parameters, suggesting that He–Ne laser maintained Chl fluorescence characteristics of plants and efficiently protected wheat seedlings against UV-B-induced injury. In addition, He–Ne laser alone showed favorable effects on light reaction kinetics associated with PSI and PSII.

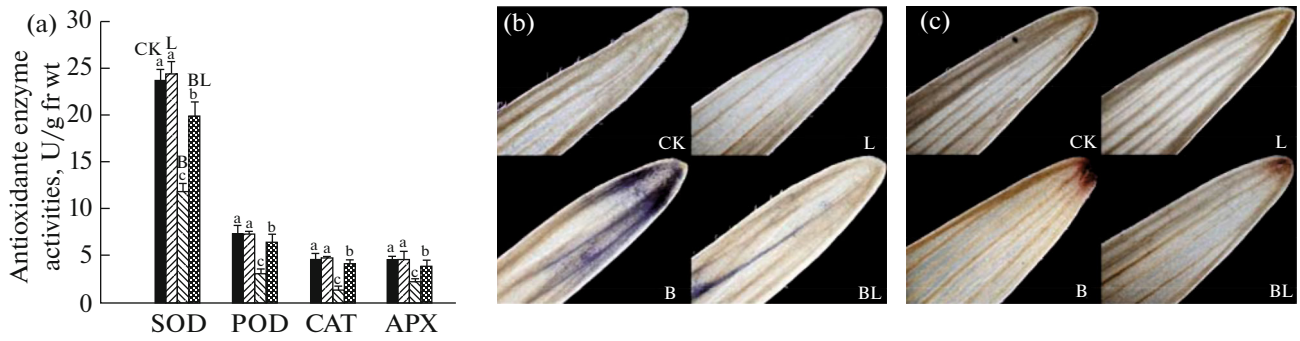

Fig. 6. Antioxidant enzyme activities and ROS levels of wheat seedlings: (a) antioxidant enzyme activities; (b) intracellular O_2^- accumulation in leaves; (c) intracellular H_2O_2 accumulation in leaves. SOD—superoxide dismutase; POD—peroxidase; CAT—catalase; APX—ascorbate peroxidase; CK—control wheat seedlings without any stress or other treatment; L—plants radiated by He–Ne laser for 4 min d^{-1} (without UV-B radiation); B—plants stressed with enhanced UV-B for 8 h d^{-1}; BL—plants with combined treatment of enhanced UV-B stress for 8 h d^{-1} and He–Ne laser for 4 min d^{-1}. Treatments with different letters indicate significantly different at $P < 0.05$. Values represent means ± SD calculated from 5 replicated measurements for each treatment, $n = 5$.

The reduction in the ratio of Fv/Fm and Y(II) indicated that photosynthetic electron transport was disturbed, and thylakoid membrane structure in the donor side of PSII was damaged by UV-B stress. The change of Fv/Fm and Y(II) induced by He–Ne laser suggested that the laser showed efficient protective effects for PSII structure and the cooperativity of PSII reaction centers. A possible mechanism is that He–Ne laser alleviated the loss of PSII function through regulating the biosynthesis of atrazine-binding polypeptide. Non-photochemical quenching (qN and NPQ) values present heat dissipation of photosynthetic apparatus, the photochemical quenching (qL) parameter indicated the fraction of open PSII centre in plants, whereas photochemical quenching (qP) is usually used to evaluate energy dissipation via PSII electron transport. Our data showed that enhanced UV-B stress resulted in a significant reduction of photo-protection for photosynthetic apparatus, as indicated by significant decreases in qN and NPQ values. The reductions of qL and qP values further suggested that the decreased photo-protection was induced by UV-B stress due to the destruction of PSII reaction centre. However, He–Ne laser illumination could ameliorate the inhibitory effects on physiological activities of PSII induced by UV-B stress.

Our results showed that Y(NA) and Y(I) in wheat subjected to enhanced UV-B stress were significantly decreased, and Y(ND) was significantly increased, as compared to plants subjected to a combination with He–Ne laser and UV-B stress. Furthermore, the present study also demonstrated that a lower Y(I) resulted in the reduction in the electron transport rate of PSI (ETRI), implying that PSI photochemical efficiency was distinctly limited and PSI function was more severely damaged by UV-B stress. However, He-Ne laser illumination could significantly alleviate deleterious effects of UV-B stress on wheat seedlings PSI.

In this study, we speculated that enhancement effects of laser on photosynthetic pigment biosynthesis capacity, and Chl fluorescence yield of photosynthetic apparatus was most likely attributed to plant antioxidant system. We found that He–Ne laser illumination significantly alleviated the inhibitory effects of enhanced UV-B stress on antioxidant enzyme activities, and decreased intracellular ROS accumulation. So we assumed that there are correlations between plant antioxidant system and the protective effects of He–Ne laser on photochemical impairments of photosynthetic apparatus of wheat seedlings. However, the precise interactions and mechanisms between plant antioxidant system and Chl fluorescence require further study in future.

Preliminary studies reported that protection mechanism of He–Ne laser on plants should be attributed to its electromagnetic effects due to little heat and pressure emitted from the laser [12, 14]. Another explanation on its biological effects is that plants irradiated with the laser absorb more energy than the controls, and the laser energy triggers physiological processes and biochemical metabolism due to the transformation of light energy to chemical energy. Consequently, in our study, the physiological processes and biochemical metabolism related with photosynthesis of plants illuminated with He–Ne laser were accelerated, then photosynthetic pigment biosynthesis capacity and antioxidant enzyme activities were enhanced, ROS cytotoxicity was detoxified that caused the revert of Chl fluorescence characteristics, and finally photosynthetic efficiency was also augmented notably (Fig. 7). However, the interactive correlations between plant oxidant/antioxidant balance and Chl fluorescence characteristics are thus thor-

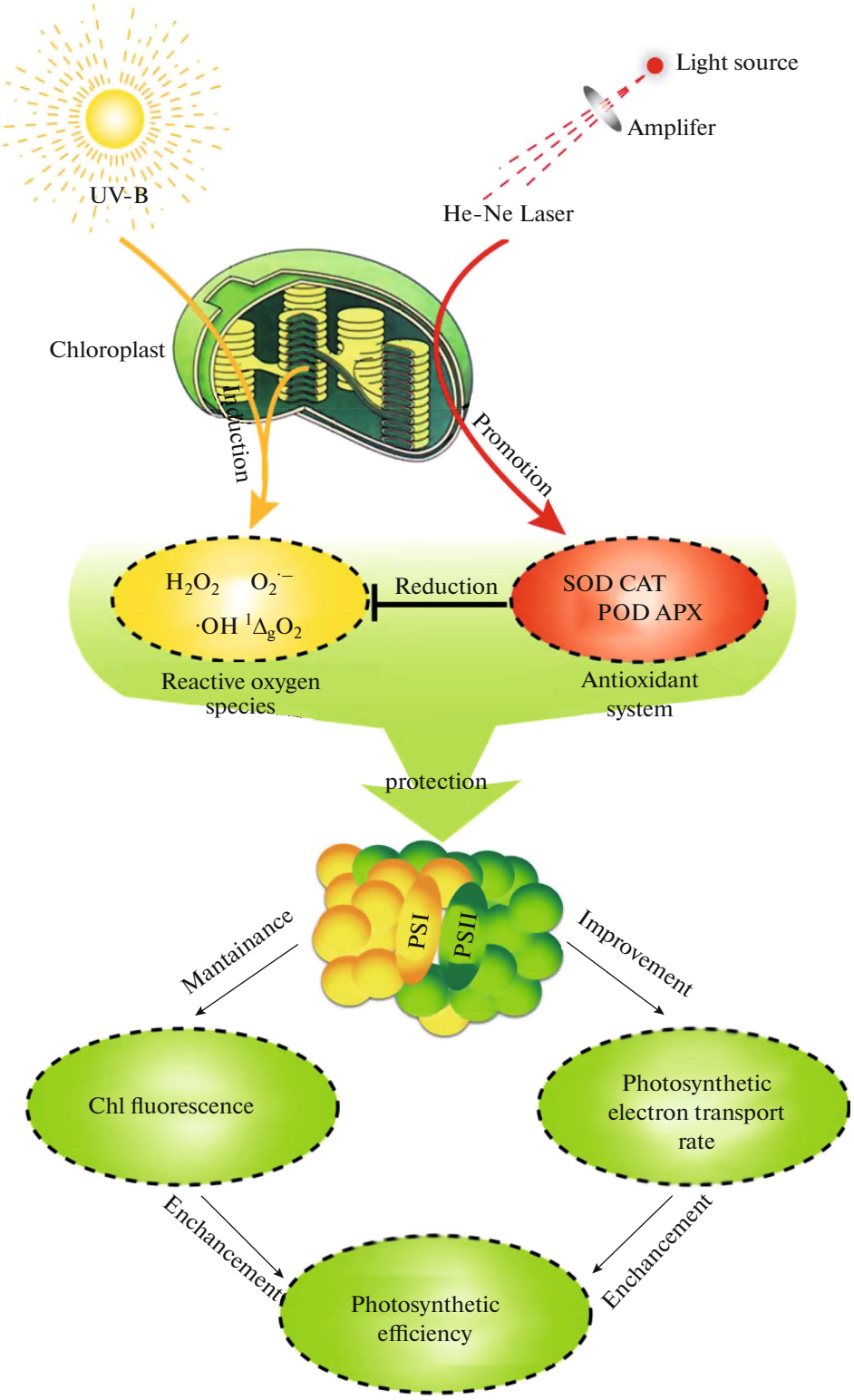

Fig. 7. Putative schematic of He—Ne laser illumination protects wheat seedlings against photochemical impairment caused by enhanced UV-B stress. He—Ne laser induces plant antioxidant system that detoxifies the cytotoxicity of the excessive ROS releasing from chloroplast, then protects PSI and PSII against photochemical impairments, finally improves photochemical activities and enhanced photosynthetic efficiency.

oughly unknown. Therefore, in vitro studies such as ours are necessary for generating mechanistic insights into the specific biological effects of He–Ne laser on photochemical characteristics of agricultural plants.

ACKNOWLEDGMENT

This research work was supported by Shanxi Province Nature Science Foundation, project no. 2014011028-5 and Shanxi Natural Foundation of Shanxi Normal University, project no. ZR1414.

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