He-Ne laser treatment improves the photosynthetic efficiency of wheat exposed to enhanced UV-B radiation

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Abstract
The level of ultraviolet-B (UV-B) radiation on the Earth’s surface has increased due to depletion of the ozone layer. Here, we explored the effects of continuous wave He-Ne laser irradiation (632 nm, 5 mW mm\(^{-2}\); 2 min \(d\)^{-1}) on the physiological indexes of wheat seedlings exposed to enhanced UV-B radiation (10 KJ m\(^{-2}\) \(d\)^{-1}) at the early growth stages. Wheat seedlings were irradiated with enhanced UV-B, He-Ne laser treatment or a combination of the two. Enhanced UV-B radiation had deleterious effects on wheat photosynthesis parameters including photosystem II (chlorophyll content, Hill reaction, chlorophyll fluorescence parameters, electron transport rate (ETR), and yield), the thylakoid (optical absorption ability, cyclic photophosphorylation, Mg\(^{2+}\)-ATPase, and Ca\(^{2+}\)-ATPase) and some enzymes in the dark reaction (phosphoenolpyruvate carboxylase (PEPC), carbonic anhydrase (CA), malic dehydrogenase (MDH), and chlorophyllase). These parameters were improved in UV-B-exposed wheat treated with He-Ne laser irradiation; the parameters were near control levels and the enzyme activities increased, suggesting that He-Ne laser treatment partially alleviates the injury caused by enhanced UV-B irradiation. Furthermore, the use of He-Ne laser alone had a favourable effect on seedling photosynthesis compared with the control. Therefore, He-Ne laser irradiation can enhance the adaptation capacity of crops.

Keywords: He-Ne laser, abiotic stress, wheat

(Some figures may appear in colour only in the online journal)
cell death in plants [10]. Therefore, identifying a method to protect plants from enhanced UV-B irradiation is important, but few such protection methods are widely employed.

Compared with chemical methods for plant UV-B protection, physical methods are more effective and beneficial. Low energy laser irradiation of plants is an environmentally friendly method for accelerating plant growth [11]. The use of low power continuous wave He-Ne laser irradiation of plants has recently been explored. He-Ne laser treatment has considerable biological effects on seed metabolism [12]. Suitable laser pretreatment of embryos enhances drought stress resistance in wheat seedlings by decreasing the concentrations of MDA and H$_2$O$_2$ [13]. Treatment with He-Ne laser helps accelerate seedling growth and development [14], and can repair UV-B-induced damage and shorten the recovery time [15, 16].

In addition, He-Ne laser can repair damage induced by osmotic stress in wheat [17]. These observations suggest that laser has a positive physiological effect on seedling growth under abiotic stress [18].

Here, we examined the effects of He-Ne laser treatment on the photosynthesis of wheat seedlings exposed to enhanced UV-B. First, we selected the optimal doses of enhanced UV-B radiation and He-Ne laser treatment by measuring parameters in wheat such as root length, seedling height, and MDA, soluble protein and sugar contents in response to treatment. Then, we subjected wheat seedlings to four different treatments, including the control (CK), single enhanced UV-B (B) treatment, He-Ne laser (L) treatment, and combined He-Ne laser and enhanced UV-B treatment (BL). We examined photosynthetic factors in the treated seedlings, such as chloroplast fluorescence parameters and enzyme activities during cyclic photophosphorylation and the dark reaction.

Thus, the primary objective of this investigation was to explore the effects of continuous wave He-Ne laser irradiation on the photosynthesis of wheat seedlings exposed to enhanced UV-B radiation at the early growth stages.

### 2. Materials and methods

Wheat seeds (*Triticum aestivum* L. cv. JIN-8) were selected and sterilised for 10 min with 1% NaClO, and then washed for 10 min with running water. Thirty seeds were cultured on wet filter paper per Petri dish in a growth chamber at 25 °C and 60% relative humidity, and watered daily. There were four treatments with three replications. The day after the seeds germinated, irradiation treatment was applied according to the light/dark period shown in table 1. The inhibitory effects of enhanced UV-B and He-Ne laser treatment were dose dependent. An enhanced UV-B radiation dose of 10.0 KJ m$^{-2}$ d$^{-1}$ has great biological significance because higher doses of enhanced UV-B stopped wheat growth, making treatment was presented). The detection of soluble sugar and protein content followed Jagendorf’s protocol [19].

#### 2.2. Measurement of chlorophyll fluorescence parameters

The optical densities of thylakoids were measured from 400 to 750 nm by fluorescence spectroscopy (JASCO FP-8000). Photophosphorylase, Mg$^{2+}$-ATPase and Ca$^{2+}$-ATPase activities were detected using the methods of Jagendorf [19].

#### 2.3. Measurement of thylakoid parameters

PEPC enzyme was extracted according to Sayre [21] and its activity was detected according to Blanke [22]. MDH and CA were measured using the methods of Souss [23] and Wilbur [24], respectively. Chlorophyllase (chlace) was extracted as described by Minguez [25], and its activity was measured as described by Amir [26].

#### 2.4. Measurement of enzyme activities in the photosynthetic carbon assimilation process

Data analysis was performed with Sigma-plot (version 10.0) and Origin (version 8.0). The least significant difference test at the 5% probability level was applied to test the differences among mean values of each attribute.

### 3. Results

Wheat seedlings treated with different doses of enhanced UV-B radiation and He-Ne laser exhibited considerable changes in plant height, root length and MDA, and soluble sugar and soluble protein contents. The results of treatment of wheat seedlings with different doses of enhanced UV-B radiation are shown in figure 1. The inhibition of these parameters by enhanced UV-B treatment was dose dependent. An enhanced UV-B radiation dose of 10.0 KJ m$^{-2}$ d$^{-1}$ has great biological significance because higher doses of enhanced UV-B stopped wheat growth, making
this dose impractical for further experiments. The most efficient dose of enhanced UV-B should decrease the density of O₃ by 20% [27]. Therefore, we used this dose for subsequent experiments. During the process of selecting efficient doses of He-Ne laser treatment, we found that low doses of this treatment have positive effects on wheat plant height, root length, and soluble sugar and soluble protein levels (figure 2). However, with increasing irradiation time, He-Ne laser inhibited wheat growth and damaged wheat seedlings at the physiological level. The results show that H₂ is the most efficient dose, i.e., irradiating wheat seedlings for 2 min at 5 mW mm⁻².

Wheat seedlings treated with the selected doses of enhanced UV-B and He-Ne laser were divided into four groups. Figure 3 and table 2 show the growth and biomass
of 7-d-old wheat seedlings under different treatments. The growth of wheat seedlings exposed to enhanced UV-B radiation was inhibited, but seedlings developed after being irradiated by He-Ne laser. In the combined enhanced UV-B and He-Ne laser treatment group, the damage from UV-B irradiation had been alleviated.

The effects of different treatments on chloroplasts are shown in figure 4. The chloroplast parameters became more conducive to plant growth after irradiation with He-Ne laser. After treatment with enhanced UV-B, the chloroplast membrane was damaged (a), the contents of chloroplast proteins (b) and chlorophyll a, b (c, d) were reduced, and the activities of CF1 ATPase (e) and the Hill reaction (f) were significantly reduced compared with the control \((P < 0.05)\). In the combined enhanced UV-B and He-Ne laser treatment group, these parameters were near control levels and were significantly different from those of the enhanced UV-B treatment group. As the treatment time increased, the trend remained as follows: \(L > CK > BL > B\). The effects of He-Ne laser and enhanced UV-B radiation on wheat chlorophyll fluorescence parameters exhibited a similar trend \((g, h, i)\). These results indicate that He-Ne laser has a positive effect on chloroplasts and alleviates the damage caused by enhanced UV-B irradiation.

A similar trend was observed for the role of He-Ne laser treatment on the photosynthetic electron transfer chain in wheat (figure 5). As the wheat seedlings exposed to enhanced UV-B radiation grew, the yield \((a)\) and ETR \((b)\) declined, but the trend remained \(L > CK > BL > B\). That is, the activity of the PSII reaction centre was altered by He-Ne treatment. The qP \((c)\) and qN \((d)\) reflect the quantum yield of primary photochemistry. These values were affected by enhanced UV-B but were near control levels after He-Ne laser irradiation. These results indicate that He-Ne laser treatment can repair the damage to the electron transfer chain caused by exposure to enhanced UV-B.

Moreover, thylakoid parameters (figure 6), including optical density \((a)\) and the activities of cyclic photophosphorylation \((b)\), Mg\(^{2+}\)-ATPase \((c)\), and Ca\(^{2+}\)-ATPase \((d)\), decreased after exposure to enhanced UV-B. However, these reductions were less pronounced in the combined enhanced UV-B and He-Ne laser treatment group. As the wheat seedlings developed, the trend was maintained as \(L > CK > BL > B\). These results indicate that He-Ne laser repairs the damage to wheat thylakoids caused by enhanced UV-B radiation.

The activities of enzymes involved in carbon assimilation in wheat seedlings in different treatment groups are shown in figure 7. Enhanced UV-B radiation had negative effects on the activities of PEPC \((a)\) and CA \((b)\), while it improved the activities of MDH \((c)\) and chlase \((d)\). He-Ne laser treatment had positive effects on the activities of these enzymes. In the combined treatment group, these activities were near control levels and were significantly different from those of the enhanced UV-B treatment group.

### 4. Discussion

Photosynthesis is one of the most sensitive metabolic processes in plants. Since photosynthesis is directly linked to biomass production and yield, it is essential to study the response of photosynthesis to UV-B stress [6]. The UV-B-sensitive sites of the PSII complex are shown in figure 8 [6, 28]; this information may help spark ideas for protecting plants from enhanced UV-B radiation.

Recently, the impact of He-Ne laser on plant growth has received increasing attention, which has led to some preliminary studies [12, 17, 29–31]. However, previous reports have not focused on the thorough analysis of the positive, restorative effects of He-Ne laser treatment on plant photosynthesis. Here, we used He-Ne laser treatment to repair the damage to the plant photosynthetic system caused by exposure to enhanced UV-B radiation.

It is widely accepted that various reactive oxygen species (ROS) induced by UV-B radiation function as both signalling and damaging agents [32]. As a result of ROS production, higher amounts of oxidative membrane damage are produced.
by UV-B irradiation [33]. In this study, the chloroplast membrane was damaged and chloroplast protein levels reduced by exposure to enhanced UV-B radiation (figures 4(a) and (b)). This result indicates that enhanced UV-B radiation may affect reactions that lead to solute fluxes. After seedlings were irradiated with He-Ne laser, the chloroplast protein content increased, but the mechanism underlying this process has not been reported. Perhaps laser activation affects the metabolic activity of the chloroplast, leading to an increase in its protein content.

The water-oxidising complex (OEC) appears to be sensitive to UV-B radiation [34]. Hill reaction activity reflects the activity of the OEC. In this study, He-Ne laser partially recovered the activity of the Hill reaction, which was inhibited under enhanced UV-B radiation (figure 4(f)). He-Ne laser may affect the manganese cluster in the OEC of PS II and improve the activity of the OEC. The activity of CF1-ATPase was also reduced by exposure to enhanced UV-B; that is, the activity of cyclic photophosphorylation declined (figure 4(e)). A previous study showed that ATPase activity depends on
This result is that the He-Ne laser activates the antioxidant enzymes (MDH, chlase). A possible reason for this is that the He-Ne laser activates the antioxidant enzymes (PEPC, CA) but increased the activities of photosynthetic products [39]. PEPC is a key enzyme in biosynthetic reactions whose activities depend on the accumulation of photosynthetic products [40]. In this study, the activities of PEPC and CA were reduced upon exposure to enhanced UV-B radiation (figure 7). He-Ne laser inhibited the respiratory enzymes (PEPC, CA) but increased the activities of photosynthetic enzymes (MDH, chlase). A possible reason for this result is that the He-Ne laser activates the antioxidant defence system to remove ROS, whose main sources include chloroplasts and mitochondria [41]. Low doses of laser in the visible range appear as small amounts of heat and pressure. The biologically positive effects of He-Ne laser might be due to electromagnetism [1, 14–16]. The main effect of this treatment may be energy absorption by the plant at the molecular level, which would affect plant physiology.

5. Conclusion

This study explored the use of He-Ne laser treatment to alleviate the effects of enhanced UV-B radiation on wheat photosynthesis. He-Ne laser irradiation stimulated the activities of key enzymes and altered various parameters involved in wheat seedling photosynthesis. Single He-Ne laser irradiation yielded improved morphological parameters, while the combined He-Ne laser and enhanced UV-B radiation group exhibited rehabilitation effects. However, more studies are needed to appraise the positive role of He-Ne laser on other abiotic stresses. Ultimately, perhaps He-Ne laser will be commonly applied under field conditions to increase the yield of wheat crops.

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