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Effects of CO_2 laser pretreatment on drought stress resistance in wheat

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Abstract

In order to determine the role of laser in drought stress resistance of spring wheat (*Triticum aestivum* L.), seed embryos were exposed to CO_2 laser radiation for 0 min, 1 min, 3 min and 5 min, respectively, and when the seedlings were 12 days old they were treated with 10% (w/v) PEG6000 solution for 10 days. Changes in the concentration of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), glutathione (GSH), ascorbate (AsA), oxidized glutathione (GSSG), carotenoid, zeaxanthin, the production rate of superoxide radical (O_2^-), the activities of ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GP), glutathione-*S*-transferase (GST) and the growth parameters of seedlings (plant height, leaf area and dry weight) were measured to test the effects of laser pretreatment. The results showed that suitable laser pretreatment of embryos enhanced drought stress resistance in wheat seedlings by decreasing the concentration of MDA and H_2O_2, GSSG, the production rate of O_2^-, leaf area and increasing the activities of APX, GST, GP and POD and AsA, carotenoid and zeaxanthin concentration. It is suggested that those changes in MDA, O_2^- H_2O_2, anti-oxidative enzymes and anti-oxidative compounds were responsible for the increase in drought stress resistance observed in the experiments. The results also showed that the laser had a long-term positive physiological effect on the growth of drought stress seedlings. This is the first investigation reporting the use of CO_2 laser pretreatment to enhance drought stress resistance of spring wheat.

Keywords: CO_2 laser; Wheat (*Triticum aestivum* L.); Drought stress; Anti-oxidative enzymes; Anti-oxidative compounds

1. Introduction

Drought is by far the leading environmental stress-limiting crop yields world-wide [1,2] and recent global climate change has made this situation more serious [3]. Average wheat yield throughout the world is only 30–60% of the attainable yield potential because of water shortage [4]. Drought invariably leads to reactive oxygen species (ROS) generation in plants. The generation of ROS is one of the earliest biochemical responses of eukaryotic cells to biotic and abiotic stresses. ROS are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids [5,6]. However, plants are endowed with an array of antioxidant enzymes and molecules such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), glutathione reductase (GR, EC 1.6.4.2), ascorbate (AsA) and glutathione (GSH) to cope with ROS [7]. Evidences suggest that drought causes the oxidative damage through enhanced generation of ROS and an inefficient response of antioxidant defense system in plants [5,8,9].

Laser was used widely as pre-sowing seed treatments to increase seed germination and seedling growth [10–12]. Previous studies indicated that suitable doses of laser

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irradiation improved plant metabolism, enzymatic activities and the concentration of chlorophyll [12,13]. Qi et al. [13–15] and Han et al. [16,17] have also illustrated that laser pretreatment could protect cells of broad bean and wheat from enhanced UV-B radiation damage, and have a repair role on the seedlings of broad bean damaged by enhanced UV-B. The long-term physiological effect of He–Ne laser treatment on UV-B damaged plants was also observed [14]. Many studies found that laser pretreatment could alleviate the inhibitory effect of UV-B radiation, and delayed the senescence process [14]. In these studies, CO_2 laser could attenuate the oxidative stress induced by enhanced UV-B radiation. However, little is known about the role of laser pretreatment in protecting plant from drought stress damage. On the basis of our previous works, we hypothesized that CO_2 laser pretreatment could improve the drought stress tolerance of crops.

In this study, we used wheat (*Triticum aestivum* L.) species as experimental material to examine the effect of seed pretreatment by CO_2 laser on protecting wheat from drought stress damage. Our aims were to (1) determine the combined effect of CO_2 laser pretreatment and drought stress on wheat seedlings; (2) evaluate the effect of seed pretreatment by CO_2 laser on protecting wheat from drought stress damage using plant morphological and physiological indices.

2. Materials and methods

2.1. Plant materials

Spring wheat (*T. aestivum* L. cv. Mianyang 26) seeds were selected for uniform size. Spring wheat seeds were obtained from Yangling Breeding Center of National Wheat Engineering Research Center of China.

2.2. Laser treatment

A CO_2 laser (wavelength 10,600 nm, power density 20.1 mW mm^{-2}, beam diameter 5 mm) directly irradiated the embryo of a spring wheat seed for 1 min, 3 min and 5 min, respectively. Five replications of 40 pure seeds were used for each of the different irradiated time. One seed was pretreated only once by laser irradiation. The seeds were exposed to laser one by one. No laser irradiation was regarded as the control (CK). The CO_2 laser (Model No.: MSHCO2-A-C800MM) was made in Northwest University (China).

2.3. Drought stress

After CO_2 laser pretreatment, seeds were sterilized for 10 min by 0.1% $HgCl_2$ and were washed for 50 min by flowing water. They were grown in Petri dish (diameter 18 cm), flushed daily with a modified half-strength Hoagland's solution, in a growth chamber under a 12 h photoperiod at 250 μmol m^{-2} s^{-1}, 70% relative humidity and 25 °C/18 °C (day/night). When the seedlings were 12 days old, they were treated with 10% (w/v) PEG6000 solution for 10 days. 10% (w/v) PEG6000 treatment was chosen according to our previous work (data not shown). On the 2d, 4d, 6d, 8d, 10d of drought stress, leaves were sampled respectively for various analyses.

2.4. Effects test

2.4.1. MDA determination

MDA concentration was measured according to Predieri et al. [18]. Samples of leaves (0.30 g fresh weight, FW) were homogenized in 50 mM phosphate buffer (pH 7.8), and then centrifuged for 15 min at 8000*g*. A 1 mL supernatant sample was combined with 2.5 mL thiobarbituric acid (TBA) and incubated in boiling water for 20 min and then quickly cooled in an ice-bath. The mixture was centrifuged at 10,000*g* for 5 min and the absorbance of supernatant was monitored at 532 and 600 nm. After subtracting the non-specific absorbance (600 nm), the MDA concentration was determined by its molar extinction coefficient (155 mM^{-1} cm^{-1}) and the results expressed as μmol MDA g^{-1} FW.

2.4.2. Hydrogen peroxide (H_2O_2) determination

Hydrogen peroxide concentration was estimated by the modified method according to Shi et al. [19]. Leaf samples (0.5 g fresh weight, FW) were homogenized in an ice bath with 3% (w/v) trichloroacetic acid. The homogenate was centrifuged at 12,000*g* for 15 min, and 1 mL of supernatant was added to 1 mL of 100 mM potassium phosphate buffer (pH 7.0) and 2 mL of 1 M KI. The absorbance was measured at 390 nm. The content of H_2O_2 was calculated based on a standard curve.

2.4.3. The production rate of O_2^- determination

The production rate of O_2^- was measured by the modified method as described by Elstner and Heupel [20]. Fresh leaves (0.20 g) were homogenized in 1 mL of 50 mM phosphate buffer (pH 7.8), and the homogenate was centrifuged at 10,000*g* for 10 min. Then 0.5 mL of the supernatant was added to 0.5 mL 50 mM phosphate buffer (pH 7.8) and 0.1 mL of 10 mM hydroxylamine hydrochloride. After 1 h reaction at 25 °C, the mixture was added 1 mL of 17 mM sulfanilamide and 1 mL 7 mM α-naphthylamine at 25 °C for 20 min, the specific absorbance at 530 nm was determined. Sodium nitrite was used as standard solution to calculate the production rate of O_2^-.

2.4.4. Enzyme activity determination

Frozen leaves (0.20 g) were homogenized in a mortar and pestle with 2 mL of 50 mM ice-cold phosphate buffer (pH 7.8) containing 1 mM EDTA. The homogenate was centrifuged at 15,000*g* for 15 min at 4 °C. The supernatant was used for assays of the activities of SOD, POD, CAT, GST and APX. All operations were carried out at 4 °C.