Ecotoxicology and Environmental Safety 92 (2013) 320–326

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of selenium on agronomical characters of winter wheat exposed to enhanced ultraviolet-B

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ARTICLE INFO

Article history:
Received 16 December 2012
Received in revised form
15 March 2013
Accepted 18 March 2013
Available online 16 April 2013

Keywords:
Nutritional quality
Selenium
UV-B
Winter wheat
Yield

ABSTRACT

Enhanced ultraviolet-B (UV-B) is one of the most important abiotic stress factors that can influence almost every aspect of plant. Selenium (Se) can increase the tolerance of plants to stressful environment. The paper mainly reported the effects of enhanced UV-B, Se supply and their combination on agronomical characters of winter wheat under field conditions. Enhanced UV-B caused a marked decrease in chlorophyll content, plant height, spike length, weight per spike, grain yield and protein content, grain nitrogen (N) and iron (Fe) concentration, and increased hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and proline content, and grain zinc (Zn) and manganese (Mn) concentration under without supplemental Se supply. However, it also decreased plant height, spike length, weight per spike, grain yield and Fe concentration, and increased H_2O_2 content, grain potassium (K), Zn and Mn concentration under supplemental Se supply. On the other hand, Se supply induced an evident increase in chlorophyll content, spike length, weight per spike, grain yield, grain protein content, grain N, Fe, copper (Cu), and Se concentration under both UV-B levels. Moreover, significant UV-B × Se interaction was found on plant height, chlorophyll, MDA, H_2O_2 and proline content, and grain protein, N, K, Cu and Mn concentrations in wheat. The obtained results supported the hypothesis that Se supply increased the yield and improved the quality of winter wheat exposed to enhanced UV-B to some extent.

1. Introduction

In spite of the current efforts to restrict the production of ozone depleting substances, thinning of the stratospheric ozone layer and increasing penetration of ultraviolet-B (UV-B) radiation to the earth's surface will still continue for decades (De la Rose et al., 2001). Enhanced UV-B radiation has had a profound impact on the production activities of human society, and the impacts on agricultural production were greater and more direct.

Enhanced UV-B is one of the most important abiotic stress factors that influences almost every aspect of plant growth (Yao and Liu, 2007a; Singh et al., 2011a; Eichholz et al., 2012). It has been reported to reduce plant height, the number of tillers, net photosynthetic rate, biomass and yield, and eventually change crop quality (Zheng et al., 2003; Gao et al., 2009; Singh et al., 2011b). Research has suggested that harmful effects of UV-B radiation on plants were often a consequence of reactive oxygen species (ROS) production (Strid et al., 1994), which eventually resulted in oxidative stress in plant. Hence, searching for suitable ameliorants or stress alleviant is very important for agriculture production (Kong et al., 2005; Yao et al., 2011; Liu et al., 2012).

Recent researchers have identified that selenium (Se) could not only promote growth and development of the plant, but also increase resistance and antioxidant capacity of the plant subjected to stress, although Se is not considered to be required by higher plants (Hartikainen and Xue, 1999; Terry et al., 2000; Yao et al., 2011). Kong et al. (2005) reported that an appropriate concentration of exogenous Se positively increased the antioxidant and osmoregulatory capacity and the salt-resistance in sorrel seedlings. Chu et al. (2010) and Hawrylak-Nowak et al. (2010) reported that exogenous Se alleviated the harmful effects of short-term cold stress on wheat and cucumber seedlings respectively. In previous paper, we have also reported that appropriate amount of Se could promote the growth of wheat seedlings, reduce ROS concentration and membrane lipid peroxidation, and increase the antioxidant ability of wheat seedlings exposed to enhanced UV-B radiation (Yao et al., 2011). The results were similar to the previous findings on ryegrass, lettuce, strawberries and buckwheat (Hartikainen et al., 2000; Xue et al., 2001; Valkama et al., 2003; Breznik et al., 2005). However, these experiments were carried out mainly on seedlings, and the results were obtained from short-term studies. In addition, previous studies mainly involved plant growth, morphological and physiological parameters. However, the

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http://dx.doi.org/10.1016/j.ecoenv.2013.03.024

ultimate goal of agricultural production is to increase yield and quality of crop. Whether Se could ultimately alleviate the effects of enhanced UV-B radiation on yield and quality of wheat crop, was a key scientific issue and worthy of study for current and future agricultural production.

Wheat is one of the most important cultivated crops in the world. Enhanced UV-B has influenced its yield and quality at present. The paper studied effects of Se, enhanced UV-B, and their combination on physiological traits, yield and nutritional quality of winter wheat under field conditions. We hypothesize that Se supply modifies the adverse effects of UV-B on winter wheat, in order to better defense the damage of enhanced UV-B on winter wheat.

2. Materials and methods

2.1. Plant material and experimental design

The field experiment was conducted in Baoding, Hebei Province. The soil had the following properties: texture loamy-meadow-cinnamon soil, organic matter 14.35 g kg^{-1}, available nitrogen (N) 55.38 mg kg^{-1}, available phosphorus (P) 22.35 mg kg^{-1}, available potassium (K) 120.32 mg kg^{-1}, and total Se 325 μg kg^{-1}. N, P and K fertilizers were added to the soil as base fertilizers before sowing to meet the nutrient demand of wheat growth; the amount was 120 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1}. The selected seeds of wheat (*Triticum aestivum* L. cv Shimai 15) from Baoding agricultural market were disinfected by immersion in 2.5% solution of sodium hypochlorite for 5 min, and sown on October 15, 2011. Wheat was sampled during the harvest season in July 2012, and some physiological parameters in flag leaves were investigated during wheat filling stage.

The experiment was a 2 × 2 factorial design with four replications. Plot size was 2 m × 2 m. The experiment consisted of four treatments in the paper: (1) ambient UV-B without supplemental Se supply (control, CK); (2) ambient UV-B with supplemental Se supply (Se); (3) enhanced UV-B without supplemental Se supply (UV-B); and (4) enhanced UV-B with supplemental Se supply (UV-B+Se).

2.2. UV-B and Se treatments

Supplementary UV-B was produced by UV-B fluorescent lamps (40 W, 305 nm, Beijing Electronic Resource Institute, Beijing, China) mounted in metal frames. The distance from the lamps to plant apex was about 60 cm and kept constant throughout the experiment. In ambient UV-B treatment, UV-B from the lamps was excluded by wrapping the tubes with 0.125 mm polyester film (Chenguang Research Institute of Chemical Industry, China), which transmits UV-A. In enhanced UV-B treatment, lamps were wrapped with 0.10 mm cellulose diacetate film, which transmits both UV-B and UV-A. The spectral irradiance from the lamps was determined with an Optronics Model 742 (Optronics Laboratory Inc., Orlando, FL) spectroradiometer, and was weighted according to the generalized plant action spectrum and normalized at 300 nm to obtain effective radiation ($UV\text{-}B_{BE}$) (Caldwell, 1971). The supplemental $UV\text{-}B_{BE}$ dose was 2.54 KJ m^{-2} day^{-1} (a 30% elevation above ambient $UV\text{-}B_{BE}$) in addition to the effective 8.45 KJ m^{-2} day^{-1} $UV\text{-}B_{BE}$ (ambient $UV\text{-}B_{BE}$). Seedlings were irradiated for 8 h (from 8:00 to 17:00) daily centered on the solar noon. The treatments began at regreening stage of winter wheat.

Extra Se (Na_2SeO_3) was sprayed at a concentration of 30 mg Se kg^{-1} solution at regreening stage of winter wheat (before UV-B treatment). Spraying volume per hectare was 1000 L. The spraying time was between 5 and 6 pm on windless sunny day. The treatments without Se were sprayed with the same amount of distilled water.

2.3. Photosynthetic pigments

0.5 g leaves were ground in 80% acetone for determination of chlorophyll content according to Lichtenthaler (1987).

2.4. Hydrogen peroxide and malondialdehyde content

Hydrogen peroxide (H_2O_2) content was determined according to Prochazkova et al. (2001). 0.5 g sample was ground with 5 mL cooled acetone in a cold room (10 °C). Mixture was filtered with filter paper followed by the addition of 2 mL 5% titanium sulfate and 5 mL ammonium solution to precipitate the titanium–hydrogen peroxide complex. The reaction mixture was centrifuged at 10 000 × g for 10 min. The precipitate was dissolved in 5 mL 2 mol L^{-1} H_2SO_4 and then recentrifuged. The absorbance of supernatant was recorded at 415 nm.

The degree of lipid peroxidation in leaf tissue was assessed by malondialdehyde (MDA) content. MDA content was determined by the thiobarbituric acid (TBA) reaction. 0.5 g leaves were homogenized with 5 mL of 20% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 3500 × g for 20 min. To 2 mL of the aliquot of the supernatant, 2 mL of 20% TCA containing 0.5% (w/v) TBA and 100 μL 4% (w/v) butylated hydroxytoluene in ethanol were added. The mixture was heated at 95 °C for 30 min and then quickly cooled on ice. The contents were centrifuged at 10 000 × g for 15 min and the absorbance was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using an extinction coefficient of 155 $mmol^{-1}$ cm^{-1}. Results were expressed as μmol g^{-1} FW.

2.5. Winter wheat yield and yield component factors

Yield components factors (plant height, spike length and spike weight) were measured on 30 randomly collected whole winter wheat plants before harvest. The wheat plants were cut with the help of pruning shears, and all the spikes in each plot were collected. Whole grains were cleaned, weighed and stored in a dry room. Wheat grain yield was expressed as kg hm^{-2}.

2.6. Starch and protein concentration in wheat grains

Starch concentration was analyzed by State Standard of the People's Republic of China (GB 5006-85). A 2.5 g sample was wet with 60 mL calcium chloride–acetic acid solution and shaken until fully dispersed. Then the sample was boiled for 30 min on glycerin solution. After the sample cooled, it was diluted to 100 mL with distilled water. 1 mL zinc sulfate solution and potassium ferrocyanide each was added to the sample and fully shaken. The supernatant was filtered and measured by polarimeter.

Total protein concentration in wheat grain was determined as total N concentration multiplied by the factor 5.7 (Teller, 1932). The protein content was converted to dry matter and expressed in percent.

2.7. Macro- and micro-element analyses

2.7.1. Nitrogen (N), phosphorus (P) and potassium (K) elements

The dry samples of wheat grains were ground with grinding mill. About 0.3 g sample was digested with 5 mL concentrated sulfuric acid for 30 min at about 300 °C, cooled for 10 min, three drops of 30% hydrogen peroxide (H_2O_2) were added and the sample was boiled for 15 min. If the sample was not colorless, the last step was repeated. After the sample cooled, it was diluted to 50 mL with distilled water and shaken thoroughly. The solutions were used for the determination of the concentration of the nutrients elements N, P and K. The measurement of N concentration was determined by using the Kjeldahl method; P was determined by the phosphovanado-molybdate colorimetry method with a spectrophotometer. K was determined by Atomic Absorption Spectrometer. Nutrient elements concentration in wheat grain was expressed as g kg^{-1} dry weight (DW).

2.7.2. Iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) elements

About 0.4 g sample was ashed in a muffle furnace at 500 °C for 24 h. After the sample cooled, the ash was dissolved in 2 mL (1:1) HNO_3, and transferred quantitatively to 50 mL volumetric flask. Fe, Zn, Cu and Mn concentration were determined by the Atomic Absorption Spectrometer. Nutrient element concentration in wheat grains was expressed as mg kg^{-1} DW.

2.8. Se element

About 0.5 g sample was digested with 10 mL of a 4: 1 mixture of HNO_3 and $HClO_4$ at 150 °C for 2 h. After the digested solution became colorless, and cooled, 5 mL of HCl (1: 1) was added to it to reduce Se (VI) to Se (IV). This process took 30 min until the sample was completely mineralized, after which it was transferred quantitatively to 50 mL volumetric flask. Se concentration was determined by atomic fluorescence spectrometry (AFS-830a, Beijing Jitian, Beijing, China). The measurement conditions were as follows: the voltage, HCl cathode current, carrier gas flow rate, the shielding gas flow rate, atomizer height, injection volume, reading time and delayed time were 280 V, 80 mA, 300 mL min^{-1}, 500 mL min^{-1}, 8 mm, 0.5 mL, 10 s and 1 s, respectively.

2.9. Statistical analysis

Analyses were performed with the Software Statistical Package for the Social Science (SPSS) version 11.0. Homogeneity of variance was tested using the Levene test prior to analysis. A two-way analysis of variance was used to determine the main effects of enhanced UV-B radiation, Se supply and their interactions on