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Ambient UV-B radiation inhibits the growth and physiology of *Brassica napus* L. on the Qinghai-Tibetan plateau

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ABSTRACT

Increased amounts of ultraviolet-B (UV-B) radiation reaching the Earth's surface through stratospheric ozone depletion are expected to have a negative effect on plant responses. However, the reliability of extrapolating indoor experiments to infer plant responses under field conditions has been questioned. Here, we report the growth and physiological responses of *Brassica napus* L. crops grown on the Qinghai-Tibetan plateau to different levels of ambient UV-B radiation (100%, 70%, and 25%). Here, we aimed to obtain a realistic evaluation of the effect of high UV-B radiation on *B. napus* L. crops in this region. We used three experimental groups: control (ambient UV-B radiation), T1 (25% exclusion of solar UV-B), and T2 (70% exclusion of solar UV-B). Compared to the control, exclusion of solar UV-B radiation enhanced specific leaf weight (SLW) and caused plant height increased with a significant increase in biomass. Ambient UV-B radiation caused the UV-B absorbing compounds of the leaves to increase, while chlorophyll a, b, and (a + b) content decreased. No significant differences in carotenoid content were detected among the three groups. Compared to the control, exclusion of solar UV-B radiation reduced antioxidant enzyme activity. Moreover, the results showed that exposure to UV-B radiation caused *B. napus* to (i) increase UV-B absorbing compounds to reduce the transmittance of UV photons through the leaf tissue, (ii) enhance antioxidant enzyme activity to scavenge reactive oxygen species (ROS), and (iii) increase carotenoids to prevent oxidative damage. However, the bleaching of chlorophyll a and damage to the photosynthetic apparatus by solar UV-B radiation caused a reduction in the photosynthetic rate.

1. Introduction

Solar ultraviolet radiation is a fraction of the solar electromagnetic spectrum that is generally divided into three classes: UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (≤280 nm). The level of UV-A reaching the Earth's surface is independent of ozone concentration, because it is not attenuated by ozone, and it causes negligible damage to biological systems (Caldwell and Flint, 1997; Solomon, 2008). In contrast, UV-C is highly energetic and extremely damaging to biological systems (Staehelin et al., 2001). Yet, ozone and oxygen in the stratosphere can strongly absorb UV-C and remove from sunlight reaching the Earth's surface (de Gruijl and van der Leun, 2000). The level of UV-B radiation reaching the Earth's surface is mainly influenced by the stratospheric ozone, which is the primary UV-B absorbing component in the atmosphere. Ninety percent of UV-B is directly absorbed by the ozone (Staehelin et al., 2001; McKenzie et al., 2007).

The depletion of stratospheric ozone results from the emission of human-made chemicals (e.g., chlorine and bromine compounds), leading to enhanced levels of solar UV-B irradiation reaching the Earth's surface (de Gruijl and van der Leun, 2000; Rowland, 2006). Since the 1970s, there has been great concern about ozone depletion and its consequences on the biosphere, because lower ozone concentrations lead to increased exposure to harmful solar UV-B (Madronich et al., 1998). Although UV-B only represents a fraction of the solar spectrum, it may exert substantial photobiological effects when absorbed by important macromolecules, such as proteins and nucleic acids (Ries et al., 2000; Watanabe et al., 2006). The increment of UV-B may affect terrestrial vegetation and ecosystems, causing a harmful effect on plant growth (Mackerness, 2000; Mpoloka, 2008). Numerous studies have demonstrated that the negative effects of increased UV-B are associated with a reduction in net photosynthesis, damage to photosystem II (PS II), and a

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decrease in chlorophyll concentrations, among other effects (Xiong, 2001; Albert et al., 2008). However, most of these studies were conducted in growth chambers and greenhouses under conditions that did not reflect the natural environment, in addition to using controls that elevated UV-B and with low background UV-A and visible light (Xu and Sullivan, 2010). Therefore, differences noted among studies might be attributed to the experimental conditions of these evaluations, as both growth chambers and greenhouse experiments were conducted, which might lead to different responses (Searles et al., 2001). Consequently, the degree of harm that UV-B causes to plant growth remains under discussion. Of concern, important changes in future crop production could be associated with plant growth and development under increased UV-B irradiation, particularly if crop plants are sensitive to enhanced UV-B irradiation (Hakala et al., 2002; Gao et al., 2003; Hidema and Kumagai, 2006). To obtain an accurate evaluation of how UV-B will affect crop plants, the experiments of the current study were conducted under field conditions.

Researchers that contributed to the World Climate Research Programme's project on stratospheric processes suggested that maximal ozone and peak UV-B levels will occur over the next decade, with a return to pre-1980 levels of stratospheric ozone and UV-B by the middle of this century (Andrady et al., 2009, 2010). However, the same researchers warn that the complicating effect of greenhouse gases might also lengthen the process. Recent evidence indicated that there is also a significant upward trend in solar UV-B irradiation at middle and high latitudes in the northern hemisphere (Schrope, 2000; Andrady et al., 2010). This phenomenon is particularly important for the Qinghai-Tibetan plateau, which is known as the "Roof of the World," and, as such, is the most sensitive region to the global climate change (Wang et al., 2006; Shimono et al., 2010). The mean daily UV-B doses in this region are higher than those of other areas at similar latitudes. Bian (2009) showed that the amount of ozone over the Qinghai-Tibetan plateau has noticeably decreased in the last few decades. Consequently, the plants that grow on the Tibetan plateau are exposed to an environment with higher solar UV-B radiation than the surrounding regions.

Our research group has previously evaluated physiological and photosynthetic responses of various alpine plants (including *Gentiana straminea*, *Microula sikkimensis*, and *Saussurea nigrescens*) under supplemented UV-B radiation field conditions in the Qinghai-Tibetan plateau (Shi et al., 2004). Although the negative effect of elevated UV-B on alpine plants has already been reported (Day and Neale, 2002; Lutz et al., 2005), the adverse effect of ambient UV-B on crop plants and agricultural systems in the Qinghai-Tibetan plateau has received limited attention. Yet, *Brassica napus* is the most extensively grown oil crop on the plateau. Thus, the future productivity of this area could be challenged by both (i) the anticipated increase in UV-B levels and (ii) the sensitivity of *B. napus* to higher UV-B radiation.

Here, we designed a field experiment to obtain a realistic evaluation of the effects of ambient UV radiation on *B. napus* crops grown under the climatic conditions of the Qinghai-Tibetan plateau. Plant responses were analyzed in terms of morphological and physiological parameters.

2. Materials and methods

2.1. Experimental site, plant material, and treatments

A field experiment was conducted at the Nursery Experimental Centre (altitude 2300 m), which is run by the Northwest Institute of Plateau Biology, Chinese Academy of Sciences, China. The center is located in the northeast part of the Tibetan plateau, and is characterized by a typical plateau continental climate that is dominated by the southeast monsoon from May to September in summer and high pressure from Siberia in winter. Summers are short and cool, while winters are long and severely cold. The mean annual temperature is 6.1 °C and mean annual precipitation is 371.2 mm, of which over 80% falls during the summer monsoon season.

Seeds of a *B. napus* cultivar (Qingza 303) were used. This cultivar is suitable for growth at altitudes below 2800 m, at which there is a short frost-free period. Seeds of uniform size and shape were selected, washed with distilled water, and sown in plots. Each treatment plot was 1.5 m × 0.8 m with three replicates of three treatments. Four border-protective rows were placed around each plot to reduce marginal effects. The substrate used for growing the seedlings was sieved topsoil from an alpine meadow. Seeds were planted in plots under UV cut off films and were exposed to natural sunlight. The UV film was wrapped around aluminum frames (1.5 × 0.8 × 0.75 height) under which the plots were maintained. The treatments included two UV-B exclusion treatments, designated as T1 (25%) and T2 (70%), and a control, designated as CK (100%). The transmission characteristics of the plastic films did not change during the experimental period. In addition, the plastic films did not emit fluorescence in the visible region. The frames received full solar radiation for most of the day without any shading, and the height of the frames was adjustable. For T1, the aluminum frames were wrapped with 0.75 mm thick cellulose diacetate (C.A.) film that reduced solar UV-B transmission by 25%. For T2, sunlight was filtered through 0.13 mm thick polyester plastic film cut off filters (Luminar, Toray Co., Tokyo, Japan), which specifically eliminate 70% solar UV-B radiation. The control (without any film wrapped around the frames) plants were grown under natural conditions. The UV-B irradiation that reached the top of the plants was measured by an ultraviolet radiation meter (Macam UV203, UK), and was checked every two days, with the height of the frames being adjusted as plants grew. The films were changed once a week to ensure uniformity in UV-B transmission.

2.2. Morphological features

After 45 days of treatment (during the early reproductive stage, when the inflorescence is visible at center of the rosette), 15 plants from each replicate were harvested and the morphological parameters were analyzed (plant height, leaf area, biomass, and specific leaf weight [SLW]). Biomass was obtained after oven drying at 80 °C for 48 h. Leaf area was determined with a portable leaf area meter (LI-COR, LI-3000, Lincoln, Nebraska, USA).

2.3. Photosynthetic pigments and UV-B absorbing compounds

Fully expanded and mature leaves were collected at the green bud stage from each frame of all replicates. Then, 45 leaf discs of 7 mm diameter were punched from the leaf blade of the leaves from the replicates of all three treatments. Then, the leaf discs were randomly divided into three groups, and were placed in bottles containing 10 ml extracting solution ($V_{\text{ethanol}} : V_{\text{acetone}} : V_{H_2O} = 45 : 45 : 1$), and were then kept in a cool and dark place for 2 weeks. The absorbance of the extracted solutions was read at 647 and 664 nm with a UV-spectrophotometer (UV-1750, Shimazu, Japan). The photosynthetic pigments were determined as described by Qaderi and Reid (2005). Another set of 45 leaf discs (also 7 mm diameter) from leaves at the green bud stage were used to extract UV-absorbing compounds in acidified methanol ($V_{\text{methanol}} : V_{H_2O} : V_{\text{HCl}} = 79 : 20 : 1$), which were also kept in a cool and dark place for 2 weeks. UV-B absorbing compounds were estimated by measuring absorbance from 250 to 400 nm with a UV-spectrophotometer (UV-1750, Shimazu, Japan). Photosynthetic pigments were estimated by