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Research Article

The Role of Reactive Oxygen Species in UV-B-inhibited Pollen Germination and Tube Growth of Canola

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ABSTRACT

During the past few decades, the stratospheric ozone reduction problem has stimulated remarkable research on higher plant responses to UV-B radiation. However, little of this work has addressed the reproductive biology of plants. The purpose of this study was to investigate the effects of UV-B radiation on reactive oxygen species (ROS) accumulation and antioxidant defense system in relation to germination and tube growth of canola (*Brassica napus* L) pollen. Our results illustrate that increased UV-B radiation decreased the pollen germination rate and tube length *in vitro*. Production of superoxide anion radical (O_2) and hydrogen peroxide (H_2O_2) increased by UV-B radiation treatment, and their accumulation resulted in lipid peroxidation. The activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were decreased by enhanced UV-B radiation. The increased ROS and lipid peroxidation, as well as decreased antioxidant activities may be attributed to the effects of UV-B radiation on pollen germination and tube growth.

Keywords: *Brassica napus L*, Pollen germination, Reactive oxygen species, UV-B radiation

ABBREVIATIONS

ANOVA: Analysis of variance; CAT: Catalase; H_2O_2: Hydrogen peroxide; MDA: Malondialdehyde; POD: Peroxidas; ROS: Reactive oxygen species; SOD: Superoxide dismutase.

INTRODUCTION

There is now little doubt that depletion of ozone in the Earth's stratosphere is occurring and that the amount of ultraviolet- B (UV-B) radiation reaching the surface of the Earth is thus increasing [1]. The stratospheric ozone layer is the key factor in reducing solar UV-B radiation reaching the earth's surface. The measurable attenuation of the stratospheric ozone layer and consequent increase in the terrestrial UV-B radiation showed a 6–14% increase since 1970s [2] which has raised interest in understanding the injurious effects of UV-B radiation on higher plants [3]. In addition, stratospheric ozone recovery may possibly be delayed due to a number of uncertainties, including interaction with other projected changes in global climate such as global warming [4]. Therefore, it still remains interesting to investigate the effects of elevated UV-B radiation on different aspects of plant growth, continuously [5].

Important indices of male gametophyte functional ability are pollen germination capacity and the rate of pollen tube growth. Pollen of open flowers appear to be well shielded from solar UV-B when still within the anther sacs, but they may be exposed to natural UV-B radiation following dehiscence until successful germination and stigma penetration occur. Moreover, pollen grain walls can transmit as much as 20% of the UV-B [6]. Therefore, most of the studies concerning UV-B radiation on reproductive biology have focused on pollen. Previous results indicated that the reproductive function was influenced by increased UV-B radiation *via* pollination [7,8]. Some studies, where pollen grains collected from healthy plants was directly exposed to UV-B by exposing the germination media to UV-B, showed that UV-B radiation has reduced pollen germination [9,10] investigated 34 taxa of pollen grains exposed to two levels of UV-B radiation. The pollen germination was inhibited in five tested species and pollen tube length in more than 50% of these species under high level UV-B radiation.

Living cells produce reactive oxygen species (ROS) under stressed and unstressed conditions creating oxidative stress leading to

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oxidative damage to lipids, proteins, nucleic acid etc. It was shown that induction of ROS production was an early effect of UV-B radiation in plants. Recently, He et al. [7] and Wang et al. [8] reported that ROS was involved in the UV-B-inhibited pollen germination and tube growth. To counter the hazardous effects of ROS, plants contain antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), as well as a wide array of nonenzymatic antioxidants. The present paper reports effects of UV-B radiation on ROS accumulation and antioxidant defense system in relation to germination and tube growth of canola (*Brassica napus L*) pollen.

MATERIAL AND METHODS

Pollen collection and irradiation

Canola (B. napus. Faclon) pollen was collected from flowers of approximately the same age. Approximately 0.1 g of pollen was collected from ~1,000 flowers (30 plants) for each biological replicate, and three biological replicates used for each analysis. Immediately after collection, pollen grains were transferred into petri dishes. The petri dishes were grouped into two and exposed to control (without UV-B radiation) and UV-B radiation treatment in the growth chamber (operated at 28°C, 400 µmol m^{-2} s^{-1} photosynthetically active radiation and 50–60% relative humidity). UV-B radiation was provided by four lamp tubes (Philips Tl 12/40w). The light was filtered with 0.13 mm thick cellulose diacetate (transmission down to 290 nm) for supplemental UV-B radiation or 0.13 nm thick polyester plastic film (absorbed all radiation below 320 nm) as control. The petri dishes were placed in the side of UV-B lamps and the desired UV-B radiation (0.5 W m^{-2}) was obtained by changing the distance between the lamps and the petri dishes. In order to get uniform exposure for all the pollen, the petri dishes were gently shaken every 5 min during the treatment process. In this study, *Brassica napus* pollen was exposed to control and UV-B radiation treatment for 0, 1, 2, 3 and 4 h. After the exposure, the pollen in each petri dish was separated into two portions. The pollen grains of one portion were inoculated in the medium for measurement of pollen germination and tube length. Another portion was stored in liquid nitrogen for enzyme analyses.

Pollen germination and tube length *in vitro*

Pollen grains were cultured in Hodgkins and Lyons media containing 9% sucrose and 13% polyethylene glycol (PEG-4000). The pollen was incubated in light and high humidity for 4 h at either 23°C or 35°C. Germinating pollen (those with pollen tubes greater than twice the length of the pollen grain) were counted and photographed using a super high quality microscopic photographs were taken using a DP70 digital camera (Olympus Optical Co., Tokyo, Japan) interfaced to a BX51 Olympus microscope (Olympus Optical Co., Ltd., Tokyo, Japan). Pollen germination was determined as a percentage of total pollen. Pollen tube length was acquired by measuring20 randomly selected pollen from each replication using a microscope.

Statistical analysis.

The germination data were tested for normality before analysis of variance. Data were analysed using SAS software (Version 9.2, SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) was performed on all results and differences between the means were compared using Duncan values (P<0.05). To evaluate the relationship among variables, they were also subjected to Pearson correlation analysis based on data matrix.

RESULTS

Both the pollen germination rate and tube length decreased as time of exposure increased in all UV-B radiation treatment (Figure 1). The pollen germination rate decreased continuously for up to 4 h under control condition. Under UV-B radiation treatment, the germination rate increased from 79% to 84.6% in the first 1 h treatment, and then decreased significantly to 47% after 2 h, 27.5% after 3 h and 10.4% after 4 h (Figure 1). The results indicate that the pollen tube length was sensitive to elevated UV-B treatment. Under control conditions, the pollen tube length was only decreased by 39% after exposure for 4 h, while under UV-B radiation treatment; it was decreased by 41% and 70% after exposure for 3 and 4 h, respectively (Figure 2).

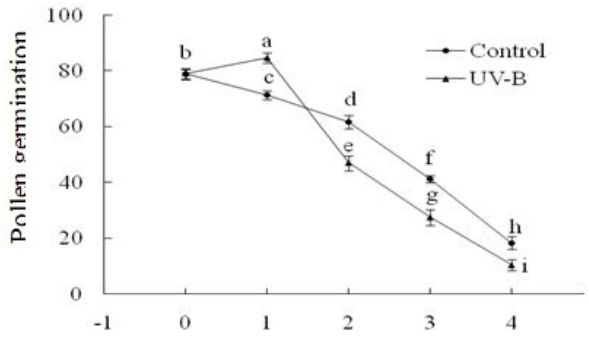

Figure 1: Effects of UV-B radiation on pollen germination and pollen tube length

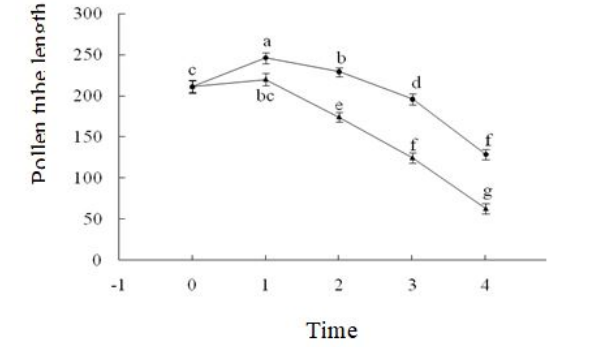

Figure 2: Effects of UV-B radiation on pollen germination of *Brassica napus*. Data are the means of three replicates ± SE. Means with different letters are significantly different at P < 0.05.

However, H_2O_2 content was more in UV-B radiation than control. Under control conditions, H_2O_2 production ranged from 23 to 45 µmol g-1 after for 4 h. While under UV-B radiation treatment, H_2O_2 production ranged from 23 to 69 µmol g-1. Also H_2O_2 production showed negative correlation with pollen germination rate (r=0.81, P<0.01) and pollen tube length (r=0.77, P<0.01) (Table1).

	PGR	PTL	MDA	O_2	H_2O_2	SOD	CAT
PTL	0.97**						
MDA	-0.86**	-0.81**					
O_2	-0.90**	-0.85**	0.96**				
H_2O_2	-0.81**	-0.77**	0.98**	0.91**			
SOD	0.77**	0.72**	-0.87**	-0.75**	-0.90**		
CAT	0.87**	0.82**	-0.87**	-0.79**	-0.88**	0.96**	
POD	0.73**	0.86**	-0.48 ns	-0.57*	-0.45 ns	0.38 ns	0.49 ns

Table 1: Correlation among pollen germination rate, pollen tube length,

reactive oxygen species accumulation, lipid peroxidation and antioxidant enzyme activities.

DISCUSSION

Role of oxidative stress due to UV-B radiation in the form of pollen germination, tube growth, ROS accumulation and antioxidant enzyme activities were studied. Results of our investigation confirmed previous reports that UV-B radiation decreased pollen germination and tube length [7,10]. In plants, several metabolic processes produce ROS, including superoxide radicals, H_2O_2, hydroxyl radicals and other free radicals. The production of ROS was substantially induced by environmental stresses. Showed that UV-B radiation induced ROS production during pollen germination. In our research, the elevated UV-B radiation increased both O_2 and H_2O_2 accumulation, which increased progressively with more time of UV-B radiation treatment. It is well known that the over production of ROS in living organisms under stress conditions is potentially toxic to the normal metabolism and results in oxidative damage, such as lipid peroxidation, protein degradation and DNA damage.

CONCLUSION

Recent studies have provided that the pollen germination and tube growth are mediated by Ca^{2+}. Flux of Ca^{2+} through the Ca^{2+}-permeable channels into a particular region of the grain where the pollen tube will emerge, or into pollen tube tip, maintains a steep tip-to-base cytoplasmic Ca^{2+} gradient, which controls pollen germination or pollen tube growth through regulation of membrane vesicle fusion and cytoskeletal dynamics, and hence abolishing the tip-to-base cytoplasmic Ca^{2+} gradient by influencing Ca^{2+} flux inhibits pollen germination and tube growth. Besides, some of studies have also shown that H_2O_2 regulates stomatal movement through the activation of Ca^{2+} channels [7]. Thus it is very interesting to know whether H_2O_2 can influence pollen germination and tube growth by mediating the Ca^{2+} channel.

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ETHICS APPROVAL

The authors completed all ethical aspects.

COMPETING INTERESTS

Study conception and design, Acquisition of data, Analysis and interpretation of data. All authors read and approved the final manuscript.

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