

Effects of UV-B Radiation on Light Signal Transduction in Wheat

Duan Jiang-yan, Niu Wen-ting and Jia Zhen-hu

College of Life Science, Shanxi Normal University, Linfen 041004, P.R. China

Abstract: In order to understand the effects of UV-B radiation on the signal transduction pathway of wheat, wheat seedlings of Jinmai 8 were treated with different doses ($10\text{KJ.m}^{-2}.\text{d}^{-1}$, $12\text{KJ.m}^{-2}.\text{d}^{-1}$ and $14\text{KJ.m}^{-2}.\text{d}^{-1}$) of UV-B radiation. Then several antagonists such as CaM antagonists, G-protein activator, Ca^{2+} antagonists, serine / threonine protein kinase were added to analyze the changes in the components of light signal transduction. By fluorescence spectroscopy and northern blotting analysis, the results showed that CaM, G-proteins, Ca^{2+} , a serine / threonine protein kinase, anthocyanin were involved in the transmission of light signaling pathway of UV-B radiation-induced wheat. Low doses and high doses of UV-B radiation induced different signaling pathway to product anthocyanin; $10\text{KJ.m}^{-2}.\text{d}^{-1}$, $12\text{KJ.m}^{-2}.\text{d}^{-1}$ doses of UV-B radiation induced the expression of chalcone synthase (CHS) gene, while $14\text{KJ.m}^{-2}.\text{d}^{-1}$ doses of UV-B radiation inhibited its gene expression. Therefore, it was concluded that low doses and high doses of UV-B radiation resulted in two different signaling pathways for regulation of plant physiology and biochemistry.

Key words: Wheat • UV-B radiation • Light signal pathway • CHS gene expression

INTRODUCTION

As the extensive use of CFCs and other halogen compounds, it have caused extensive damage on the stratospheric ozone, which absorbs solar ultraviolet and make it special material to protect ecosystem. The result of ozone depletion is to increase the UV-B radiation (UV-B) reaching the surface [1]. It is estimated that ozone have reduced by 1%, the amount of UV-B radiation increased by 2% [2]. UV-B radiation would have an effect on plant morphology, physiology, biochemistry, genetics, photomorphogenesis, etc [3-5]. Therefore, it has become a paramout research subject about plant damage and repair mechanisms by UV-B radiation.

Signal transduction refers to the process, which makes an interaction between extracellular signals and cell-surface receptors, then turns extracellular signals into intracellular signals and occurs a series of intracellular signaling cascade. In other words, biological cells react to the signals or external stimuli and adjust cell metabolism, proliferation, differentiation, functional activity and apoptosis [6]. Consequently, the research of signal transduction has certain significance.

Under the radiation of UV-B, a kind of light, plants show different morphological and physiological responses, which may involve multiple signal transduction pathways [7]. UV-B radiation mediates signal expression through cytoplasm signal transduction cascade in plants [8]. With the MAP kinase pathway induced by UV-B, plants have different morphology and reactions of physiology. This may involve multiple intracellular signal transduction pathways, including intracellular calcium, calmodulin, serine / threonine kinase and phosphates enzymes, etc, which regulates the expression of CHS (chalcone synthase) gene [9]. Different doses of UV-B radiation make different effect on plants. Many studies have found that low doses of UV-B radiation can also serve as stimulated conditions of signaling pathway, stimulating the metabolism and biological changes in the growth and development, such as bio-formaldehyde phonetic compounds synthesis, inhibition of hypocotyls extension, etc [10-13]. Whereas, high doses of UV-B radiation can inhibit the growth of soybeans and wheat, manifested in the form of dwarf plants, plant type diminution, soluble protein content decline, reactive

oxygen species content rise [14]. It was also found that strong UV-B radiation can cause plant damage even necrosis in the experiment.

Research of UV-B radiation effects on the signaling pathway is still in the data accumulation phase, even has not been reported. This paper analyzes the UV-B radiation-induced signaling pathways in wheat, participated by a variety of G-proteins, Ca^{2+} , CaM, serine/threonine protein kinase, CHS genes and discuss the different doses of UV-B radiation toward the regulation of anthocyanin content and the induction of CHS gene expression. It uses three different doses of UV-B radiation --- $10\text{KJm}^{-2}\text{d}^{-1}$, $12\text{KJm}^{-2}\text{d}^{-1}$, $14\text{KJm}^{-2}\text{d}^{-1}$ to process of wheat and adopts G-protein activator, Ca^{2+} antagonists, CaM antagonists, serine / threonine protein-kinase antagonist, then applies fluorescence spectroscopy and northern hybridization.

MATERIALS AND METHODS

Plant Materials: Wheat seeds (Jinmai 8, *Triticum aestivum*) were provided by the Institute of Shanxi Academy of wheat.

Experimental Design: Full grain, uniform size wheat grains were surface sterilized by 75% ethanol for 30s, then washed three times with sterilized water. Wheat grains were grown in Petri dishes with sterilized gauze pad, then poured B5 culture medium with 2% sucrose, 0.8% agar. 30 seeds each plate, repeat three times every treatment, placed in the light incubator.

The grains were grown with a daytime temperature of $12\text{--}31^{\circ}\text{C}$ with lightening and a nighttime temperature of $9\text{--}15^{\circ}\text{C}$ with darkness. After 3 days of germination, these grains were subjected to UV-B treatment. Different intensity UV-B radiation treatment was carried out with lightening treatment at the same time. The UV-B radiation dose of B1, B2 and B3 were 10.00, 12.00 and 14.00 $\text{KJm}^{-2}\text{d}^{-1}$, respectively, the CK group was the control with on UV-B treatment (Table 1). UV-B radiation was produce by UV-B lamp (30W, 297nm). The treatment time were 8h every day, handled a total of 7d. UV-B radiation intensity was determined with 742-type radiation detector (Optronics Laboratories Orlando, FL, USA). Antagonist and activator, such as EGTA with 1000 $\mu\text{mol/L}$, W7 with 1mmol/L, staurosporine with 50nmol/L and PTX (pertussis toxin) with 100ng/ml, were added to the cultured medium, when needed at final concentration.

Table 1: The setting and disposal program of each group

Treated group	Light (h/d)	UV-B(KJ/d)	Darkness culture (h/d)
CK	8	—	16
B1	8	10	16
B2	8	12	16
B3	8	14	16

Extraction and Measurement of CaM: The method of extraction and measurement of CaM was as reported with slight modification [15]. The mainly steps include dialyzing, salting out, centrifugalizing, phenyl-sepharose 4B. Identification of CaM used polyacrylamide gel electrophoresis. Determination of CaM was used Fluorescence spectrophotometry (LS-50B fluorescence spectrometer). The CaM content in root, stem and leaf were determined 10times, respectively. Using software packaged with the fluorescence spectrophotometer compute its average value.

Determination of Anthocyanin: The method was as reported [16]. Wheat leaves were weighed and placed in propanol:HCl:H₂O(18:1:81) extraction liquid and boiling for 3 min. Extraction were carried out at room temperature overnight. The absorbance at 535 nm and 650 nm were determined, respectively. The calculated equation was: $(A_{535}-A_{650}) / \text{g} \cdot \text{fresh weight}$. Anthocyanin content was the average value of five repeats experiment.

Analysis of CHS Gene Expression: Analysis of CHS gene expression was used northern hybridization as reported by Sambrook, 1989 [17].

RESULTS

Effects of UV-B Radiation on the Signal Transduction Pathway of Wheat

The Influence on CaM: In B1 and B2 groups of UV-B radiation, the fluorescence intensity of CaM in root, stem and leaf increased in different degree, while decreased in B3 group, indicating that low doses UV-B radiation causes an increase in CaM content, while high doses of UV-B radiation will lead to reduce CaM content. Whether or not treated by W7, the trends of CaM fluorescence intensity caused by the enhancing UV-B radiation were coincident (Fig.1). Because of the antagonism of W7, the CaM content of treatment groups is lower than that of non-W7 treatment groups generally.

CaM content increased with PTX treated (Fig.2). And the trend of PTX treated are in the same with only treated with UV-B radiation, which shown a low-dose inducement and high-dose repression of UV-B radiation for CaM synthesis.

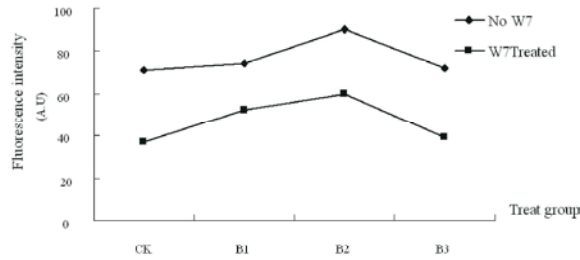


Fig. 1: Effect of W7 treated under different UV-B radiation
(CK, $0 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B1, $10 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B2, $12 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B3, $14 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$)

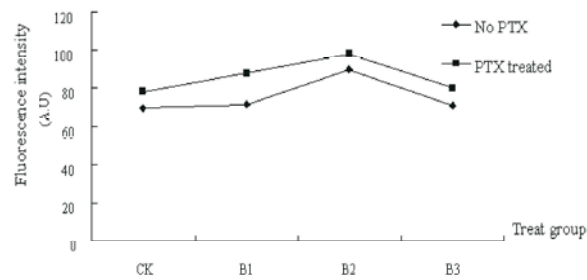


Fig. 2: The impact of different doses of UV-B radiation and PTX on CaM
(CK, $0 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B1, $10 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B2, $12 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B3, $14 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$)

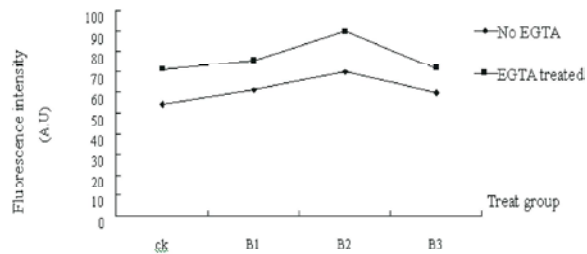


Fig. 3: The impact of different doses of UV-B radiation and EGTA on CaM
(CK, $0 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B1, $10 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B2, $12 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B3, $14 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$)

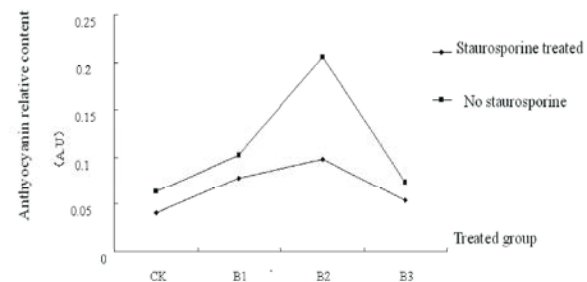


Fig. 4: Effects of different doses of UV-B radiation and staurosporine on anthocyanin
(CK, $0 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B1, $10 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B2, $12 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B3, $14 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$)

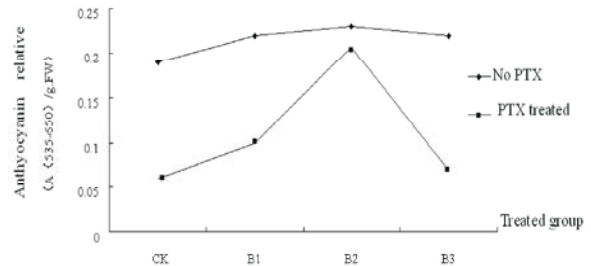


Fig. 5: Different doses of UV-B radiation and the impact of PTX on anthocyanin
(CK, $0 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B1, $10 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B2, $12 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B3, $14 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$)

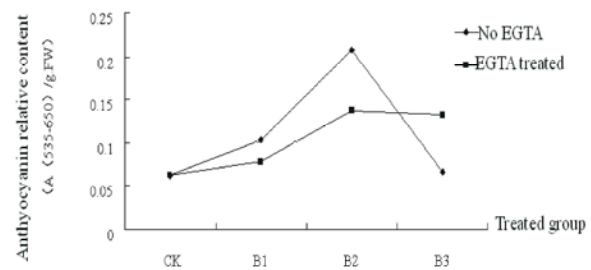


Fig. 6: Different doses of UV-B radiation and the impact of PTX on anthocyanin
(CK, $0 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B1, $10 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B2, $12 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$; B3, $14 \text{ KJ.m}^{-2} \cdot \text{d}^{-1}$)

Compared with EGTA-free group, CaM content of EGTA treated group reduced significantly (Fig. 3). Even in the EGTA environment, low-dose UV-B radiation are favorable to synthesis CaM and health-dose UV-B radiation are not helpful to synthesis CaM.

The Influence on the Content of Wheat Anthocyanin:

The anthocyanin content of every group with different doses of UV-B radiation decreased in evidence, indicating that serine / threonine protein kinase can regulate the synthesis of anthocyanin in UV-B radiation signal pathway.

Compared with staurosporine-free group, anthocyanin content of staurosporine treated group in leaves was reduced (Fig.4). This conclusion was antipodal from the groups with PTX. Compared with PTX-free group, anthocyanin content of PTX treated group in leaves was increased significantly (Fig.5). However, anthocyanin content of control groups with EGTA or W7 did not change. Anthocyanin content with 10 and $12 \text{ kJ.m}^{-2} \cdot \text{d}^{-1}$ doses of UV-B radiation was significantly reduced and with $14 \text{ kJ.m}^{-2} \cdot \text{d}^{-1}$ doses of UV-B radiation was an increase (Fig.6 and 7).

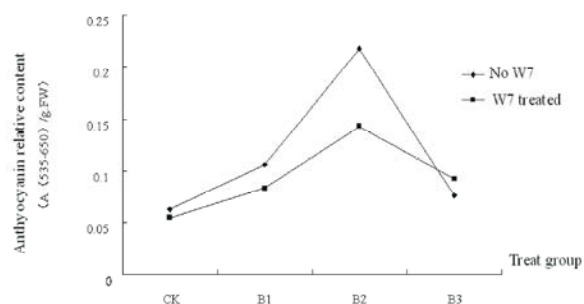


Fig. 7: Different doses of UV-B Radiation and the impact of W7 on anthocyanin (CK, $0 \text{ KJ.m}^{-2}.\text{d}^{-1}$; B1, $10 \text{ KJ.m}^{-2}.\text{d}^{-1}$; B2, $12 \text{ KJ.m}^{-2}.\text{d}^{-1}$; B3, $14 \text{ KJ.m}^{-2}.\text{d}^{-1}$)

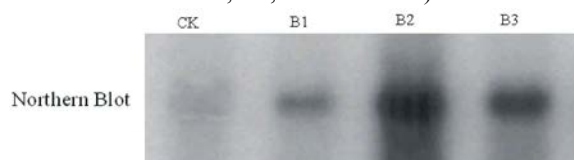


Fig. 8: The effect of different doses UV-B radiation on CHS gene expression (CK, $0 \text{ KJ.m}^{-2}.\text{d}^{-1}$; B1, $10 \text{ KJ.m}^{-2}.\text{d}^{-1}$; B2, $12 \text{ KJ.m}^{-2}.\text{d}^{-1}$; B3, $14 \text{ KJ.m}^{-2}.\text{d}^{-1}$)

Effect of Different Doses of UV-B Radiation on CHS Gene

Expression: The CHS gene expression was detected by Northern blotting (Fig. 8). The CHS gene bands were very pale in CK group and the CHS gene expression was enhanced with the UV-B radiation doses changed from B1 to B2, decreased when the UV-B radiation doses changed to B3.

DISCUSSION

Generally low doses UV-B radiation can be used as stimulated conditions, which can lead to changes in growth and development of organisms and metabolism, such as biosynthesis of phenolic compounds and inhibition of hypocotyls extension [10-12, 18, 19], while strong UV-B radiation can cause plant damage or necrosis. In this study, whether or not to join antagonists and activators, low dose UV-B indeed stimulated the expression of calmodulin and anthocyanin in wheat, high doses played an inhibitive role. And the whole present a bell-shaped curve. It was also found that the roots of wheat upturned significantly and their leaves became slender under the UV-B irradiation of $14 \text{ KJ.m}^{-2}.\text{d}^{-1}$. This phenomenon indicated the UV-B radiation of $14 \text{ KJ.m}^{-2}.\text{d}^{-1}$ can affect wheat normal physiological significantly. Jansen found that enhanced UV-B radiation

can influence photosynthesis by changing the plant itself and their leaves shape [20]. When exposed to high doses of UV-B radiation, the wheat were so damaged that the anthocyanin content and CaM decreased. This phenomenon showed that different doses of UV-B radiation can influence wheat plants through different signaling pathways.

Under low-dose UV-B radiation condition, the content of CaM and anthocyanin of antagonist treatment group less than that of the group without antagonist. It indicated that calcium ions and calmodulin were involved in the signal-regulated expression induced by low-dose UV-B radiation. However, under high-dose UV-B radiation, the anthocyanin content of antagonist treatment group is higher than that of the group without antagonist, which indicating that calcium or calmodulin did not involve in the regulation of anthocyanin content under this conditions. Brosche and Strid's study also found that there were two different signaling pathways regulating plant physiology and biochemistry under low doses of UV-B radiation and high doses of UV-B radiation conditions especially [21]. Whether high-dose UV-B or low-dose radiation can decrease the anthocyanin contents with serine/threonine protein kinase antagonist (Staurosporine) adding. It showed that serine/threonine protein kinase was involved in both the high and the low dose UV-B regulation pathway.

In many plants CaM was an important downstream target proteins of Ca^{2+} and was involved in many stress responses and physiological and biochemical functions regulation. Through the receptor of CaM on the cell, UV-B radiation can cause organism's different growth and development reaction. Parks found that UV-B radiation can act on the phytochrome receptor on the cell membrane [22]. As important components of intracellular signal transduction, CaM induced the expression of a variety of downstream target genes in cells, such as CAB gene and CHS gene, *et al* and furthermore, which affected the expression of anthocyanin and plant photosynthesis. In this study, it was found that low-dose UV-B radiation can increase CaM content, while high doses UV-B radiation inhibited the increasing of CaM content. This experiment also confirmed that Ca^{2+} was influenced by UV-B radiation and it affected CaM and various components of this pathway downstream, regulating wheat physiological and biochemical reaction.

G-protein mediated lots of signal transduction process coupled membrane receptor and played a vital role in the transmission of optical signals. It was considered to be the most upstream regulatory element in

plant optical signaling pathway. This experiment showed that in the signaling pathway induced by UV-B radiation, G-protein antagonist caused the depression of Downstream CaM and anthocyanin.

Many environment factors can change Ca^{2+} concentration in cells and the concentration of Ca^{2+} was considered an upstream regulatory element in many signal access [23]. But only a handful of documents mentioned that Ca^{2+} as an element in UV-B signaling pathway and blue light transmission [24, 25]. In this experiment, it was proved that in UV-B signaling pathway the change of Ca^{2+} concentration affected the synthesis of anthocyanin in wheat cells by using Ca^{2+} antagonists. There were two ways to increase the Ca^{2+} concentration in cells: one was extracellular Ca^{2+} entered into cells through Ca^{2+} channels on the plasma membrane, the other was calcium ions in intracellular calcium stores entered a cell through calcium ion channels on organelles. There were different intracellular calcium ion channels, such as the Ca^{2+} channels on the plasma membrane and vacuole membrane [26, 27]. The experiments have confirmed that calcium ions play a regulatory role in the UV-B radiation-mediated signaling pathway, but it still not sure that what kind of calcium channel UV-B radiation using to regulate a cell.

Many studies found that low doses UV-B radiation can stimulate plant physiological and biochemical reactions, while high doses UV-B radiation inhibit plant physiological and biochemical reactions. In this study, low dose UV-B radiation amplified the CHS gene expression, while high dose UV-B radiation suppressed CHS gene expression.

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