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EFFECT OF ENHANCED UV-B RADIATION ON WHEAT SEEDLING ROOTS

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Abstract

Enhanced UV-B radiation has multiple effects on the morphological, physiological, biochemical, and genetic mechanisms of wheat growth. The roots of wheat seedlings treated for different numbers of days with UV-B were studied. UV-B radiation had significant effects on the number of adventitious roots, average root length, root biomass, and root vigor of wheat. Whereas root showed influence of different degree with enhanced UV-B. The best method for extracting root protein among phenyl/ammonium acetate-methanol precipitation, the TCA/acetone method, and the urea/thiourea method was determined. The TCA/acetone precipitation was found to be the best method for extracting root protein by SDS-PAGE. Finally, protein from the roots of wheat exposed to UV-B was extracted using TCA/acetone precipitation. Protein content in the UV-B radiation treatment group (B) was higher than that of the control group (CK) at the early stages of the radiation treatment; with increased time of irradiation, the protein content of B was lower than that of CK.

Key words: UV-B radiation, Wheat, TCA/Acetone method, Root protein.

Introduction

Recently, UV-B radiation reaching the surface of the earth has increased because of the disruption of the ozone layer, and the potential biological effects have drawn the attention of researchers worldwide. The intensity of ultraviolet (UV) radiation has increased globally, not only in the polar regions but in other parts of the world (Chu *et al*., 2009). The effects of UV-B radiation on plants are varied. Several different cell components can directly absorb UV-B radiation, which can result in either direct or indirect effects downstream. These impacts occurring in early development can be reflected in subsequent stages of plant growth and development, such as reduced plant height and biomass accumulation (Jansen *et al*., 1998; Kakani *et al*., 2003; Ballare *et al*., 2011; Khan *et al.*, 2014), cotyledon expansion and bending (Boccalandro *et al*., 2001), and changes in leaf expansion and growth (Hopkins *et al.*, 2002; Wargent *et al*., 2009). Further, irradiating plants with UV-B light has mainly resulted in photosynthetic damage, reactive oxygen species (ROS) production, and both direct and indirect DNA damage (Jansen *et al*., 1998). Because of the sessile habit of plants, long-term adaptation to the environment and the evolution of self-protection mechanisms are necessary. For example, UV-B radiation can induce the expression and accumulation of flavonoids, anthocyanins to protect components in the cell from UV-B damage (Stapleton & Walbot, 1994; Mazza *et al.*, 2000). Overall, plants respond differently to irradiation with low or high doses of UV-B, either by stimulating protection mechanisms or by activating repair mechanisms to cope with the different levels of stress.

Wheat (*Triticum aestivum* L.) is a vital crop for China. It is also the main food crop in Shanxi Province. Several studies have shown that enhanced UV-B radiation can cause a variety of effects on wheat morphology, physiology, biochemistry, and even production (Al-Oudat *et al*., 1998; Yuan *et al.*, 2000; Kataria & Guruprasad, 2012). Plants absorb moisture and nutrition through roots; wheat has a well-developed root system with characteristics suitable for adaptation to the biotic and abiotic environment. Therefore, the effects of UV-B radiation on wheat roots were investigated in this study. Further, our results may provide a theoretical basis for the breeding of radiation resistance in wheat.

Materials and Methods

Research material: Wheat seeds (*Triticum aestivum* L.) were selected as the research materials.

Cultivation of wheat: Wheat seeds were sterilized in 1.5 % HClO for 20 min and then soaked at 37ºC in the dark until sprouting. Subsequently, sprouted wheat seeds were spread in a petri dish on a layer filter paper with the ventral groove downward and then placed at 25ºC, 55% in an Intelligent illumination incubator for cultivation.

The sprouted wheat seeds were divided into two groups, the control group (CK) and the UV-B treatment group (B), with treatment conditions presented in Table 1.

Table 1. Light/dark period of irradiation treatments.

Treatment	Light (h/d)	UV-B radiation	Dark (h/d)
CK	8	—	16
B	8	8	16

Measurement of wheat adventitious roots and root length: The roots of plants from each treatment group were photographed, the number of adventitious roots was counted, and Image J was used to measure root length. Wheat seedling root were measured on after 7 days and repeated 3 times in each group.

Determination of wheat biomass: The wet weight of wheat roots was determined on an electronic balance. The fresh plant material was placed in a paper bag and dried in an oven at 100–105°C for 10 min, and then dried to a constant weight at 70–80°C. The dried plant material was removed from the paper bags, cooled to room temperature, and weighed.

Determination of root vigor: Fluorescein diacetate (FDA) was used to detect root tip cell vitality. Three-day-old seedling roots were dyed for 3 min and washed twice. A quantitative analysis of fluorescence intensity was performed using Image J software with the polygon tool which analyzed the average density of fluorescent root meristem.

Protein extraction: The Trichloroacetic acid (TCA)/ acetone precipitation method of Damerval *et al*., (1986) was used for protein extraction with some modifications. Wheat roots (0.2 g) were ground into a fine powder in a mortar, placed in 2 mL centrifuge tubes with 2 mL of the extracting solution, and incubated overnight at -20°C. After centrifugation, the supernatant was discarded, 2 mL acetone solution (containing 0.07 % beta mercaptoethanol) pre-cooled to -20°C was added, and then the solution was precipitated for 1 h at -20°C. After centrifugation, the precipitated proteins were washed twice and treated with lysis solution.

The urea/thiourea method of Hong-Bing & Kang (2011) was also used with modifications. Wheat roots (0.2 g) were ground into a powder, 1.5 mL extracting solution was added, and the solution was centrifuged. Three times the volume of cold acetone solution was added to the supernatant which was then incubated overnight at -20°C. After centrifugation, the precipitated proteins were treated with lysis solution.

The phenol/ammonium acetate-methanol precipitation method of Hong-Bing & Kang (2011) was also performed with some modifications. Wheat roots (0.2 g) were ground into a powder, mixed with 0.5 mL extracting solution, and centrifuged, and the resulting supernatant was collected. Tris-saturated phenol was added, and the solution was incubated for 1 h at 4°C. After centrifugation, the lower phenol layer was collected, 0.5 mL extracting solution was added, and then centrifuged followed by collection of the lower phenol layer. Three times the volume of 100 mmol/L ammonium acetate methanol solution was added and incubated overnight at -20°C. After centrifugation, the precipitated proteins were treated with lysis solution.

The centrifuge conditions for all of the above three methods were 12000 g at 4°C for 15 min.

Determination of protein content in the roots of different treatment groups and SDS-PAGE analysis: Protein content was determined following the method of Jin (2012) with modifications.

Standard curve determination: Reagents were added to six test tubes as described in Table 2. Five mL coomassie brilliant blue (G-250) was added to each test tube, mixed well, and the absorbance value at 595 nm was measured 3 times for each sample.

Table 2. Reagent addition amounts for the standard curve.

Tube No	0	1	2	3	4	5
BSA/mL	0	0.2	0.4	0.6	0.8	1.0
Distilled water/mL	1.0	0.8	0.6	0.4	0.2	0
Protein content/μg	0	20	40	60	80	100

Determination of protein content: The sample extracts (20 μL), 980 μL distilled water, and 5 mL G-250 were mixed well and absorbance at 595 nm measured 3 times. Finally, the total protein content was calculated.

SDS-PAGE: Cleaning adhesive tape, glass plate and comb and so on→Make glue according to separation gel and reverse gel,and then press the surface of concrete using n-butyl alcohol→make spacer gel, reverse glue, Insert comb→insert rubber sheet in electrophoresis tank→add sample, plug the power supply →dye 3 h, then decolore.

Results

UV-B radiation effects on adventitious roots of wheat seedlings: UV-B radiation influenced the shoots and underground part of wheat seedlings. As shown in Fig. 1, increased number of irradiation days had larger influences on wheat. The CK wheat shoots grew more rapidly than those of B, and the roots of B expressed the root-bending phenomena (red arrows in Fig. 1) each day, which is consistent with previous studies. Further, UV-B radiation affected the number of adventitious roots of wheat. Little difference between CK and B roots was observed at 1 or 2 days after UV-B; however, on days 3 and 4, the number of adventitious roots was significantly greater for B than CK plants (Fig. 2). This difference may be owing to wheat root length and vigor being suppressed under UV-B radiation. The repair of damage by UV-B radiation of the plants likely gradually increased with increasing exposure to UV-B radiation, and may have even reached the limit of the ability of the plants to repair damage. Thus, by the fifth and sixth day, the number of adventitious roots in B did not differ significantly from that in CK. Finally, on the seventh day, the number of adventitious roots of CK plants was greater than that of B plants.

UV-B radiation effects on root length of wheat seedlings: As shown in Table 3 and Fig. 3, the average wheat root length of the B group on the first day was greater than that of the CK group. From the second day to the seventh day, the average wheat root length in the CK group was greater than that in the B group, with the differences being significant starting from the third day ($p \leq 0.05$). Thus, UV-B radiation exposure over a short time period appears to promote plant root growth and development. In addition, with an increase in the number of irradiation days, wheat root growth was inhibited and even ceased, which could be related to changes in the expression of wheat root proteins and root vigor under enhanced UV-B radiation. However, the requirement of water and nutrient absorption through the roots for growth could lead to the production of adventitious roots to compensate for the reduction in root length in the B group. The above result of the number of adventitious roots of wheat in the B group being greater than that of the CK group on the third and fourth days of the experiment is consistent with this interpretation.

Table 3. The influence of increasing days of UV-B radiation on wheat radicle length.

Treatment	Root mean length (cm)						
Group	1d	2d	3d	4d	5d	6d	7d
CK	0.6	1.5	3.1	4.4	5.3	7.5	10.6
B	0.7	1.4	2.1	2.8	3.5	4.5	5.4

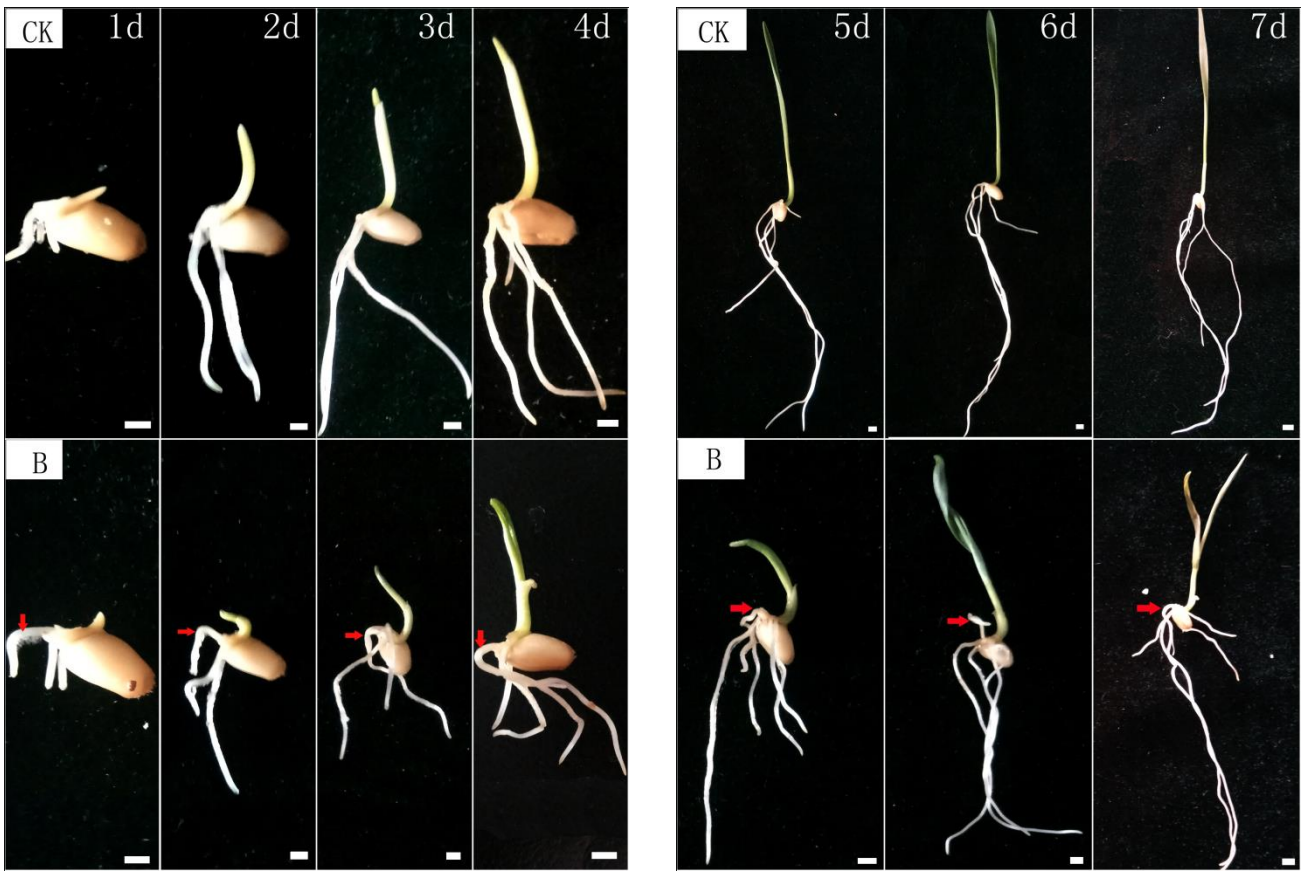

Fig. 1. The effect of UV-B radiation on wheat morphology.

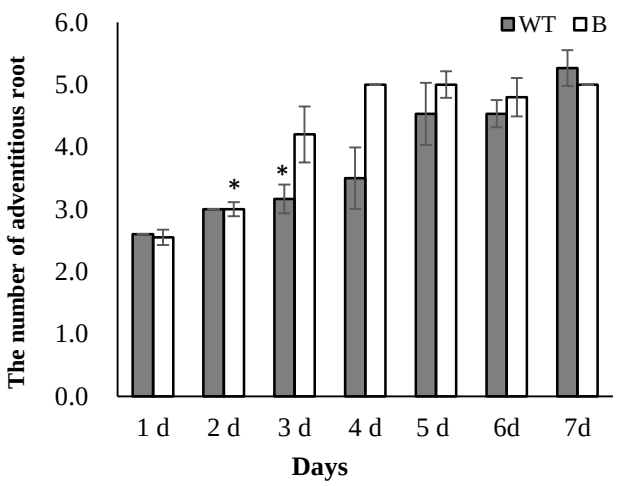

Fig. 2. UV-B radiation effect on the number of wheat adventitious roots.

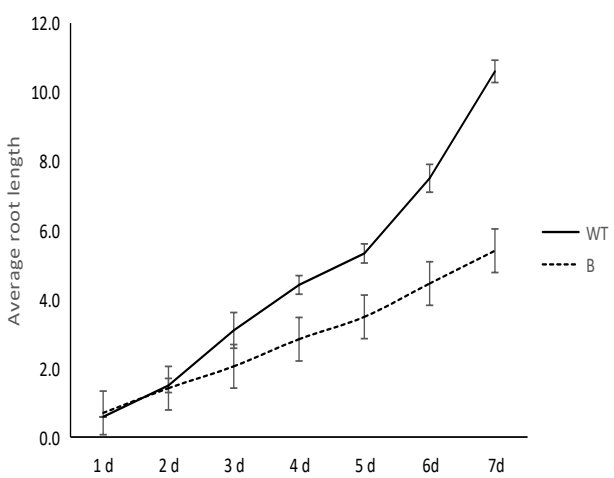

Fig. 3. UV-B radiation effects on root length of wheat seedlings.

UV-B radiation effects on wheat seedling root vigor: Wheat roots from day 3 of the experiment were stained with FDA and photographed using a fluorescent microscope (Fig. 4). The Image J software polygon tool was used to quantify root meristem fluorescence intensity to detect cell vitality of the root tips. As shown in Fig. 5, root vigor of wheat after 3 d of exposure to UV-B (B group) was significantly lower than that of the CK group ($p \leq 0.05$). Root vigor could be reduced because of effects of UV-B radiation on wheat root proteins, root auxin expression, and transportation and activity through signaling pathways.

UV-B radiation effects on root protein of wheat seedlings: Three different methods, the TCA/acetone precipitation method, phenol/ammonium acetate-methanol precipitation, and the urea/thiourea method, to extract total protein from wheat roots were compared. Based on the number of protein bands and band intensity (Fig. 6), the TCA/acetone precipitation method produced the greatest number of distinct bands. Thus, the TCA/acetone precipitation method was used to extract protein from wheat roots in the following experiments.

Fig. 4.Wheat root tips stained with FDA.

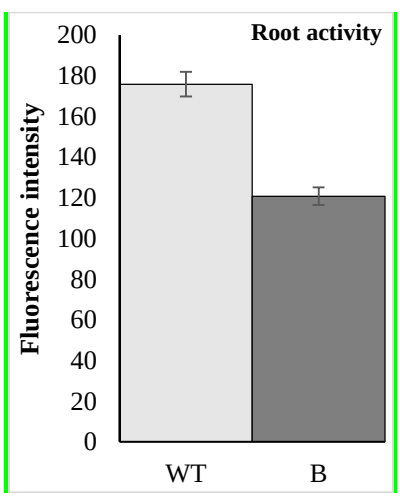

Fig. 5. UV-B radiation effects on wheat root vigor.

The TCA/acetone precipitation method was used to extract wheat root proteins on day 3, 5, and 7 of UV-B exposure (Fig. 7). Total protein content was measured with coomassie brilliant blue G-250. A standard curve was generated (Fig. 8), and the total protein content of the extracted samples was determined based on the standard curve. On the third day of UV-B treatment, wheat root protein content in the B group was significantly greater than that of the CK group (Fig. 9), which may be because short-term UV-B radiation activates root gene expression, leading to increased protein content, and ultimately, the promotion of the elongation of wheat roots. On day 5, there was not a significant difference in total protein content of the CK group and the B group; on day 7, protein content of the B group was significantly lower than that of the CK group, which is likely owing to prolonged UV-B irradiation inhibiting protein synthesis. Thus, short-term UV-B radiation promoted root protein synthesis, while long-duration radiation inhibited protein synthesis.

UV-B radiation effects on root biomass of wheat seedlings: Over the 7 days of the experiment, wheat root wet weight and dry weight of both the CK group and the B group increased (Fig. 10); however, the overall weight and rate of weight increase were lower in the B group than in the CK group, and even decreased on the seventh day. Dry weight on the first day was greater for the B group than for CK group. On the second and third days, there was no difference in dry weight between the B group and the CK group. However, from the fourth day to the seventh day, dry weight of the CK group was higher than that of the B group, with the difference being significant on the seventh day. These results showed that UV-B radiation influenced wheat root biomass, which affected the growth and development of wheat root tips.

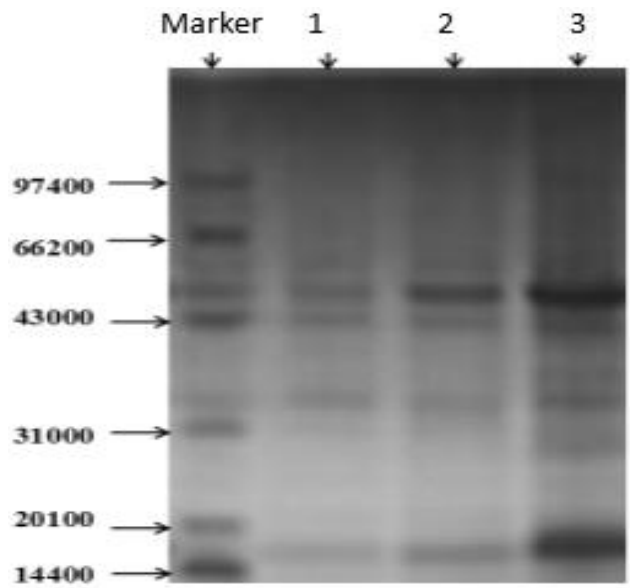

Fig. 6. Different methods of protein extraction. 1: Urea/thiourea method; 2: phenol /ammonium acetate-methanol precipitation method; 3: TCA/acetone precipitation method.

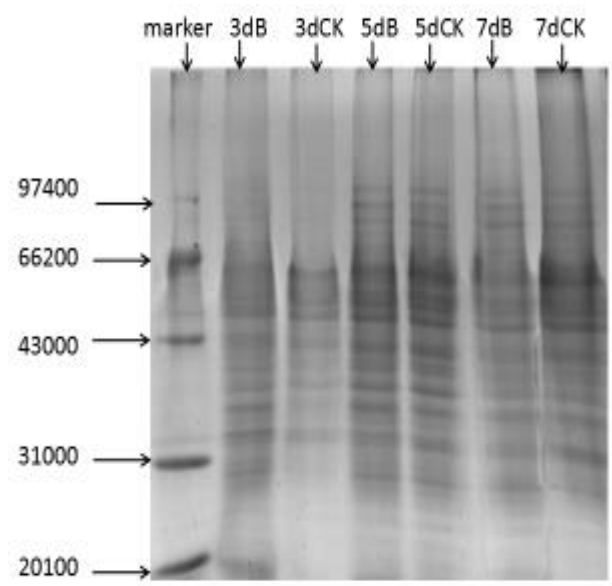

Fig. 7. The effects of different treatment days on root protein.

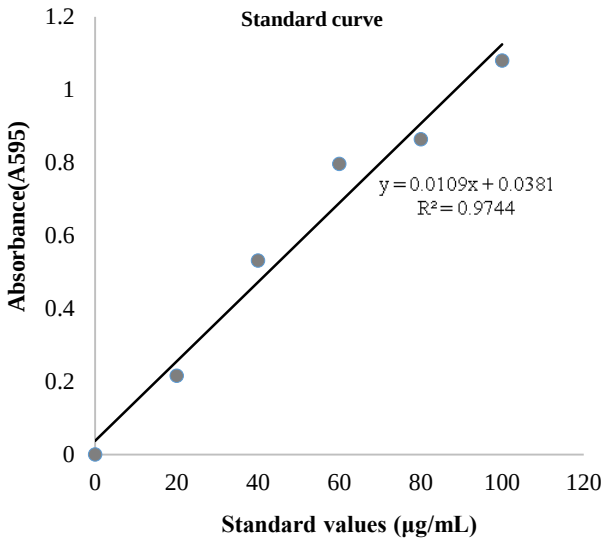

Fig. 8. Standard curve.

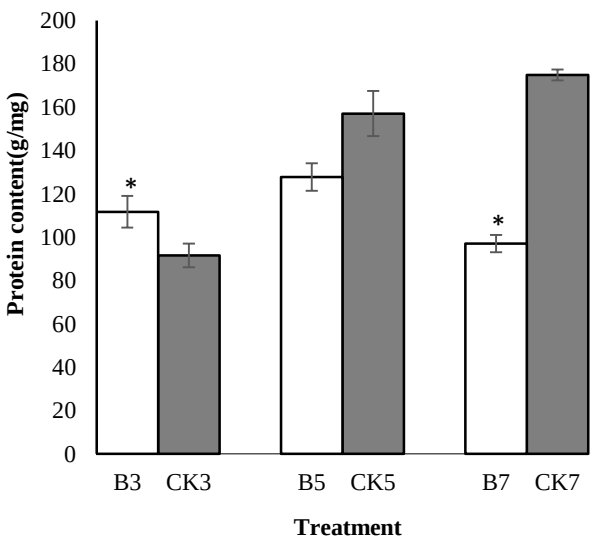

Fig. 9. The protein content of different treatments.

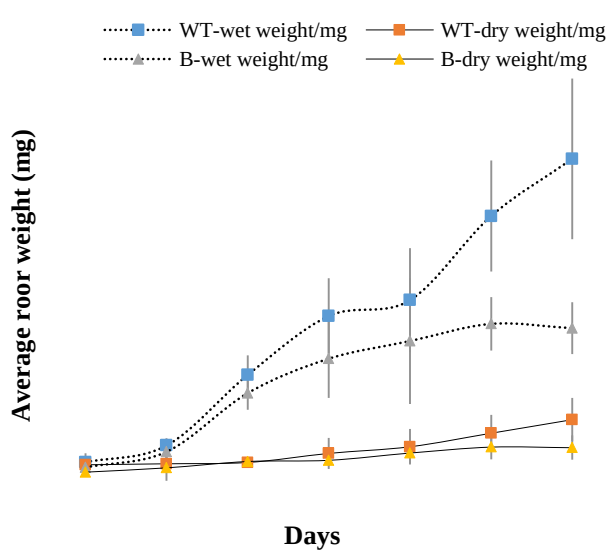

Fig. 10. UV-B radiation effects on wheat root dry weight and wet weight.

Discussion

This experiment examined wheat roots exposed to enhanced UV-B radiation. Root-bending (Fig. 1) was consistent with the findings of Han *et al*., (2007). In addition, with increased time of irradiation, UV-B radiation had different magnitudes of effects on wheat roots. The results revealed that UV-B radiation could promote an increase in the number of adventitious roots and inhibit the average growth of wheat roots. Specifically, the average root length of plants exposed to UV-B (B group) was less than the CK group (Fig. 3), which was similar to the effects of the UV-B radiation in swamps (Zaller *et al*., 2002). However, wheat seedling in the B group had to absorb water and inorganic salts for growth, which promoted an increased number of adventitious roots. More than this range, because too much accumulation of damage causing by UV-B may exceed the plant's ability to repair itself. The average root length and the number of adventitious roots did not change in the B group.

In addition, wheat root vigor, root protein content, and root biomass were studied. We speculate that UV-B radiation affected protein expression through a series of signaling pathways, which affect wheat root vigor, average root length, root-bending, and the number of adventitious roots (Fig. 11). After UV-B radiation, the wheat root biomass decreased, which was consistent with previous studies on soybeans and ryegrass exposed to UV-B radiation (Teramura & Sullivan, 1987; Wang *et al*., 2005). This result illustrates the potential of UV-B radiation to have an impact on the distribution of resources between aerial and underground plant parts and physiological signal transmission within the plant. While the growth of plant roots could be controlled by the distribution and concentration of auxin, UV - B may degrade auxin and inhibit biosynthesis, or may be the downstream molecular in the signaling pathways of UV-B radiation affected the activity of auxin-related enzymes. Further, the negative regulatory factor HY5 of auxin transport may accumulate on the side of the hypocotyl exposed to UV-B radiation leading to the plant embryo axis curving in the direction of the UV-B radiation in the model plant *Arabidopsis thaliana* (Vandenbussche & Van, 2014). Thus, the root-bending phenomenon observed under UV-B radiation may be associated with auxin transport and signaling. In addition, increasing the NO level in Arabidopsis cells could produce resistance to the influence of UV-B radiation on microtubule organization and root growth and development associated with microtubules (Krasylenko *et al*., 2012). We speculate that UV-B radiation may, therefore, rely on a series of signals to control the expression and transport of wheat root plant hormones, protein expression and activity, and NO concentration to ultimately affect the normal development of wheat roots. Signal perception may occur through UV-B receptors in the roots or signal molecules produced by UV- B radiation, but further research is required to explore these possibilities.

Three methods for extracting root proteins were compared in this study. We found that the TCA/acetone precipitation method was extremely simple and produced the most protein content with very few impurities and run adhesive results shown was the most ideal in three ways. Further, UV-B radiation influenced wheat root protein, but identifying the specific types of protein changes requires additional research.

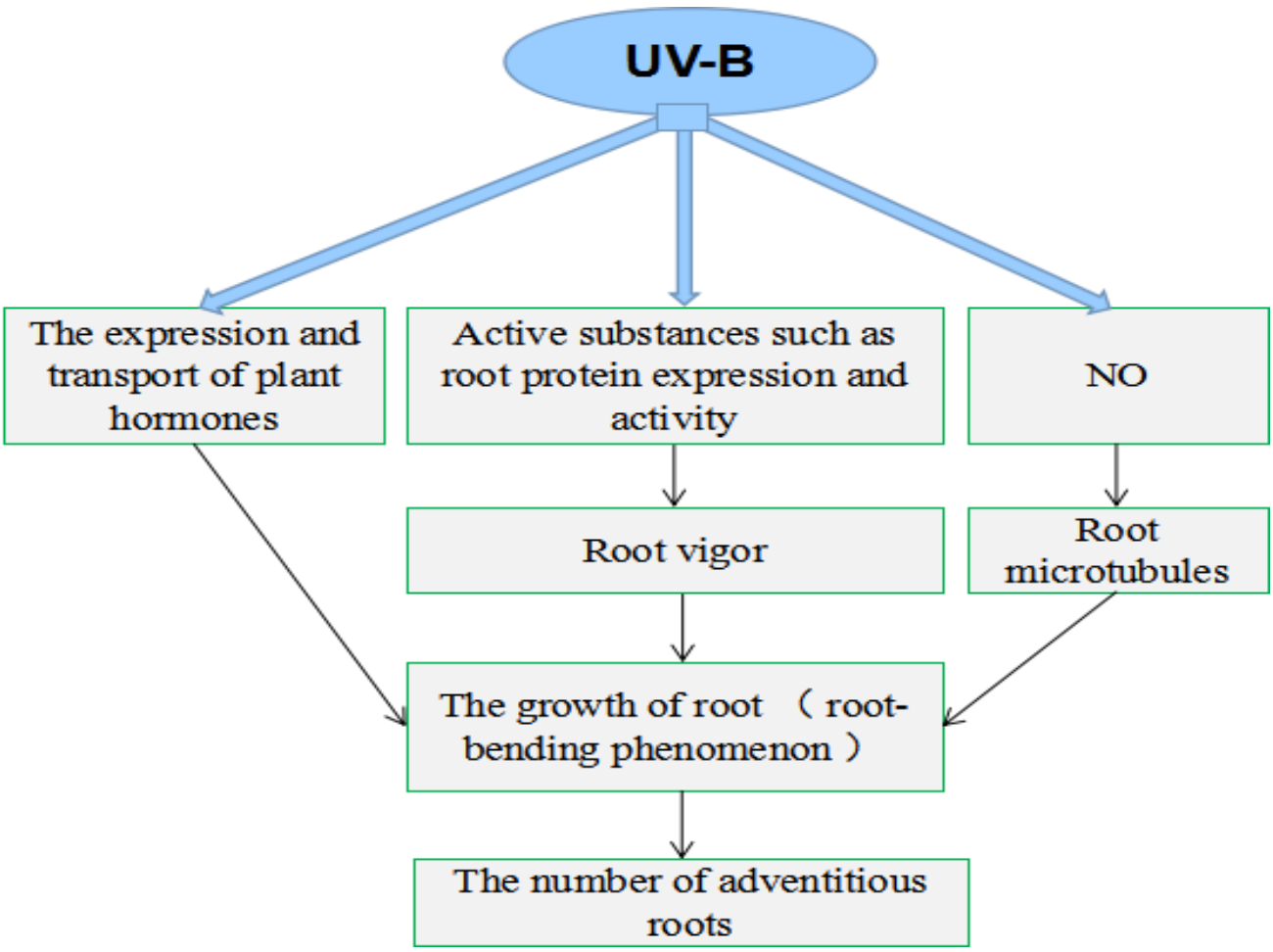

Fig. 11. UV-B radiation effects on wheat roots.

Conclusions

The wheat seedling roots responded to UV-B radiation through changes in root protein and root biomass, which decreased root vigor. In addition, the morphology of the wheat root changed to an abnormal appearance.

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