Actin filaments altered distribution in wheat (*Triticum aestivum*)
“Bending Root” to respond to enhanced Ultraviolet-B radiation

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(WITH 6 FIGURES)

Abstract

Plants adjust their shoot growth to acclimate to changing environmental factors, such as to enhanced Ultraviolet-B (UV-B) radiation. However, people have ignored that plant roots can also respond to UV-B light. Here, we find the morphology curled wheat roots under UV-B radiation, that we call, “bending roots.” The curly region is the transition zone of the root after observed at the cellular level. After exposed to enhanced UV-B radiation for 2 d (10.08 KJ/m²/d), cell size decreased and actin filaments gathered in wheat roots. We also find that H₂O₂ production increased and that content of the indole-3-acetic acid (IAA) increased remarkably. The pharmacological experiment revealed that actin filaments gathered and polymerized into bundles in the wheat root cells after irrigated H₂O₂ and IAA. These results indicated that actin filaments changed their distribution and formed the “bending root,” which was related to H₂O₂ production and increase in IAA. Overall, actin filaments in wheat root cells could be a subcellular target of UV-B radiation, and its disruption determines root morphology.

Keywords: UV-B radiation, actin dynamics, wheat, root morphogenesis.

1. Introduction

Ultraviolet-B (UV-B) radiation is a regular component of sunlight, which has a significant effect on lives (Jenkins, 2017). Almost 95% of UV-B radiation is absorbed by stratospheric ozone, but transmitted UV-B affects plant growth and development significantly (Brosche and Strid, 2003). With the decreasing of ozone and activating atmospheric circulation, transmitted UV-B radiation is increasing. Enhanced UV-B radiation damages cells’ nucleic acid and plasma membranes. It also affects photosynthesis in plant cells (Chen and Han, 2014) and induces reactive oxygen species (ROS) burst, and finally induces cell death (Nawkar et al., 2013). It is reported that a 30% increase in...
UV-B levels could seriously reduce field crop production in the USA and China (Kakani et al. 2003). However, solar UV-B radiation is now considered a light signal which can be perceived by the UV-B Resistance 8 protein (UVR8) and promote its regulatory pathway (Yang et al., 2018). The UVR8 interacts with constitutively photomorphogenic 1 (COP1), and regulates the downstream gene expression of elongated hypocotyl 5 (HY5), a transcription factor (Moriconi et al., 2018). However, previous studies have not found any other receptors like UVR8 under enhanced UV-B radiation, and know few things about plant responses to high dose UV-B radiation at the cellular level. Supplying some information on these topics is necessary for us to understand the mechanism of plant response to light.

Actin filaments are a widespread structure in cells. They contribute to the maintenance of internal architecture of the cell, drive cytoplasmic streaming (the movement of components through the cytosol), and contribute to the process of cell division (Deeks and Hussey, 2001). Actin filaments shift their patterns to respond to certain exogenous factors, including enhanced UV-B radiation (Chen and Han, 2016). Actin filament nucleation and elongation are affected by electrostatics (Crevenna et al., 2013). Previous research has found that actin polymerization is necessary to increase actin filament density and that blocking this increase with actin-disrupting drug latrunculin B leads to enhanced susceptibility of host plants to pathogenic and non-pathogenic bacteria (Henty-Ridilla et al., 2013). Actin cytoskeleton is closely related to nonhost resistance in pepperoncino against certain pathogens, and its polymerization is necessary for the expression of pepper nonhost resistance (Hao et al., 2014). Also, actin filaments play key roles in wheat resistance against Puccinia striiformis (Song et al., 2012). Under salt stress, actin filament dynamics disrupt and trigger an increase in reactive oxygen species levels in Arabidopsis roots (Liu et al., 2012). In Zea mays protodermal root cells, tungsten strongly induces cortical microfilaments which completely depolymerize; intracellular ones appeared highly bundled (Adamakis et al., 2014), while Bisphenol A (BPA) results in actin filaments with rod- and ring-like conformations (Stavropoulou et al., 2018). Under enhanced UV-B radiation, actin filaments form bundles in wheat leaves protoplasts. UV radiation can also induce rearrangement of actin filaments in human cells (Grzanka et al., 2006).

The transition zone of plant roots, which is located between the apical meristem and basal elongation region, has a unique role as the determiner of cell fate and root growth (Baluska et al., 2010). Signaling crosstalk in this region has been shown to determine developmental cell fates as well as mediate rectilinear root growth and the differential growth of tropism. Some evidence shows the root UV-B sensitive protein 1 (RUS1) and RUS2 may have some functions in UV-B signaling in roots through auxin transduction (Yokawa and Baluška, 2015). Moreover, actin filaments are thought to be a crosslinker in certain roles of cell signaling. Therefore, it is valuable information to investigate the change of actin filament dynamics in a key region such as the root.

Until now, there are few reports on how plant roots respond to enhanced UV-B radiation. Here, we focus on actin filament remodeling under enhanced UV-B radiation and formation of the bending root. The distribution of actin filaments in the transition zone was tracked after stained with phallolidin-fluorescein isothiocyanate (Ph-FITC). The plasticity of actin filaments in root transition zone and H2O2 generation were observed. These results will help us understand the possible mechanism of high dose UV-B radiation on induced plant root morphology and provide some information on the UV-B signaling pathway in plants.

2. Material and Methods

2.1. Materials cultivation

Linyou 2871 (Triticum aestivum L) kernels were supplied by the Wheat Research Institute of Agricultural Sciences in Shanxi Province, China. The seeds were selected and sterilized for 10–15 min with a 1% NaClO solution. The seeds were then washed for 10 min with running water. Thirty seeds per petri dish were cultured on wet filter paper in a growth chamber at 25 °C, 60% relative humidity, and watered daily. UV-B radiation was applied 8 h/d during the light cycle (with 8 h light and 16 h dark each day), and the intensity was 10.08 KJ/m2/d (Chen and Han, 2014). The root length was determined by a ruler. All experiments were performed with three replications.

2.2. Fluorescence staining and microscopic observation

The root tips of wheat seedlings were cut into 2 mm fragments and collected from the meristem region. 2-6 mm from to the root tip was collected and prepared as the transition zone. Double fluorescent labeling with 4',6-diamidino-2-phenylindole (DAPI) and Ph-FITC was carried out in wheat root tip cells (Luchetti et al., 2002). The incubation with Ph-FITC (50 μg/mL) was performed at 37°C for 40 min, while incubation with DAPI (100 ng/mL) was at 37°C for 10 min. The confocal microscope (Olympus FX1000) was used to observe the results; the object glass was a ×60 magnification oil immersion lens; the picture dimensions were 512 × 512 pixels. Pictures were edited with Photoshop CS6.

2.3. ROS staining in wheat seedlings roots

An aliquot of H2DCF-DA (Thermo Fisher) was added to all treatments including enhanced UV-B treatment and the control group to obtain a final concentration of 5 μM. After 20 min, samples were washed three times with PBS to remove excess H2DCF-DA and were immediately visualized using an Olympus FX1000 confocal microscope. Three independent experiments were performed.

2.4. IAA quantification

The root tips were homogenized in a cooled mortar with ice-cold 80% methanol containing 1 mM butylated hydroxytoluene (100 root tips/mL). After centrifugation at 4,6-diamidino-2-phenylindole (DAPI) and Ph-FITC were used to observe the results;
12,000 g for 10 min, samples were passed through a C18 column preconditioned with 80% methanol at 4ºC. After evaporation of methanol, samples were methylated using trimethylsilyldiazomethane in hexane at 42ºC for 30 min and diluted in 25 mM sodium phosphate buffer (pH 7.2) containing 15 mM NaCl. Quantification of IAA was performed by competitive enzyme-linked immunosorbent assay (IAA immunoassay kit Olchemin).

2.5. Pharmacological experiment

IAA was dissolved initially in dimethyl sulfoxide (DMSO) and diluted with water before irradiation. The dilution used was 1000-fold (with a final concentration of 30 nM) and the control media received an equivalent volume of DMSO. External H$_2$O$_2$ was also diluted in water and the final concentration was 10 mM. For the short-term assay, two-day-old seedlings were placed on water incubated for 2h (Tamas et al., 2014).

2.6. Statistical analyses

All experiments were carried out across three independent series with three replicates. Data results are expressed as means ± standard error (SE). Statistical significance was assessed using t-test which was performed using the SPSS 21.0 and Sigma-plot 10.0.

3. Results

3.1. The “bending root” was induced by enhanced UV-B radiation

After being exposed to enhanced UV-B radiation for 6 d, the growth of wheat seedlings was significantly inhibited (See Figure 1A). The “bending root” in the transition region can be seen after treatment by UV-B radiation after only 2 d as indicated by the white arrows in the Figure 1B. Compared with the control group (CK), the root length of 6 d old seedlings in the UV-B treatment group (B) was reduced by almost 55% (See Figure 1C) and relative elongation rate of the roots were also lessened to nearly 80% (See Figure 1D).

3.2. Cell size and the “bending root” located at the transition zone decreased

DAPI and Ph-FITC were used to stain the nucleus and cell range at the cellular level. The results showed that the “bending root” was located at the transition zone, which is the region between the apical meristem and basal elongation region.

A previous study showed that the region is full of a certain signaling pathway. Here, we found that cell size in the transition region was smaller than the control. This result indicated that enhanced UV-B radiation affected the cell expansion process. As shown in Figure 2A and 2B, the blue color derived from DAPI staining is in the enhanced UV-B treated group. The abnormal morphology indicated that the movement or the distribution of chromosomes in this area was destroyed under UV-B radiation. According to observed results, the cell size was reduced to nearly 65% compared with the control group (See Figure 2C). As previously shown in Arabidopsis studies, the “root bending” is controlled by auxin signaling. Therefore, it could be that UV-B radiation influenced auxin content. We determined the IAA content in each group, and results showed an increase of 46% in the UV-B treatment group.

**Figure 1.** Phenotype of wheat seedlings under enhanced UV-B radiation. UV-B radiation inhibited wheat seedlings growth strongly (A) and induced “bending root” as the white arrow indicated (B). The root length decreased almost 55% (C) and the relative root elongation lessened nearly 80% after exposed to enhanced UV-B radiation (D). CK is the control group, B represents UV-B treatment group. Data are means ± SE (n = 3).
compared to the control (See Figure 2D). These results allow us to infer that UV-B radiation mainly affected the cell expansion due to the increase in IAA level.

3.3. The ROS burst in the "bending root" region

As in the "bending root" region, we detected ROS levels in the "bending root." As shown in Figure 3, the fluorescent intensity of the "bending root" region is much higher, almost two-fold, than the control (See Figure 3B). This result indicated that H$_2$O$_2$ production could be a very important second messenger which activates other signaling pathways or affects other components in the cell. It may possibly be an indicator to show a plant’s response to stress.

3.4. Actin filaments gathered in the "bending root" region

The cytoskeleton is a key factor in maintaining cell morphology and mitosis. Here, the distribution of actin filaments in different treatments was observed after being stained with Ph-FITC (See Figure 4). The results showed that actin filaments gathered as bundles in the "bending root," but were unconsolidated in the control group. As mentioned above, the cell size was reduced in the
3.5. External H$_2$O$_2$ and IAA induced bundling of actin filaments

A pharmacological experiment was performed to detect the influence of ROS and IAA on the distribution of cellular actin filaments. Two regions of the root were used in the experiment. As shown in the Figure 5, the actin filaments formed actin bundles after being exposed to IAA and H$_2$O$_2$ either in the transition zone or meristem region of the wheat root. These results indicated that the change in the distribution of cellular actin filaments mainly originated from ROS and IAA content variety, with enhanced UV-B radiation as the original effector.

*Figure 3.* H$_2$O$_2$ production in the “bending root” region. H2DCF-DA was used for the H$_2$O$_2$ staining which showed in green (A). Compared with the control, the intensity showed H$_2$O$_2$ production is highly increased in the wheat root under enhanced UV-B radiation (B). CK is the control group, B represents UV-B treatment group. Data are means ± SE (n = 3).

*Figure 4.* Actin filaments gathered under UV-B treatment. All the images shown are superimposed stacks of 15 optical sections. Nucleus and actin filaments were stained blue with DAPI and green with Ph-FITC, respectively. Compared to the control (CK), the actin filaments were gathered as bundles after exposed to UV-B radiation (B). Bars =20 μm.
4. Discussion

UV-B radiation affects plant growth and development in many aspects. Especially causes a reduction in plant biomass. Therefore, it is necessary to understand the impact of this environmental factor on crop plants. Recent studies have focused on the effects of UVR8 signaling pathway and plant morphogenesis (Yang et al., 2018). But there have been fewer studies on the influence of high dose UV-B radiation on plants, especially in crops. We found that high intensity UV-B radiation regulated the dynamics of cellular actin filaments in wheat cells and lead to abnormal mitosis. Other individuals have also been concerned with the yield of crop plants exposed to enhanced UV-B radiation, which have resulted in fewer studies of the effects of UV-B radiation at the cellular level. Our results here supply this missing information on cellular processes and their response to UV-B radiation.

There is doubt surrounding how plant roots can sense light signals if the roots are underground in darkness. One possible explanation for this anomaly is that the surface of the ground is loose and light can penetrate to the root. More importantly, some photoreceptors including UVR8 are found not only in aerial parts of plants, but also in their roots (Briggs, 2014). People proposed that light is sensed by the roots directly under natural soil conditions. Specifically, light may pass through the soil to the root or may be conducted through vascular tissue to the root. Recently, it was reported that light was channeled through the stem to the roots, where it activated the red-light photoreceptor phytochrome (Lee et al., 2016), consistent with the hypothesis that the aboveground light environment is monitored by the roots by stem-piped light under natural conditions. UVR8 is expressed in roots, which indicated plant roots could sense the UV-B light as the way mentioned above. This made our study full of meaningful.

In this study, the wheat root was exposed to UV-B light with any cover and formed a “bending root.” UV-B radiation induced ROS production which damaged the root cell pectin and formed pectin-containing vesicles through endocytosis (Baluška and Mancuso, 2013). In this respect, the root apex transition zone may be very responsive to UV-B due to the high rate of pectin recycling found precisely in this root apex region (Baluška and Mancuso, 2013). In this zone, root-specific UV-B sensing proteins RUS1 and RUS2 are expressed and, more importantly, control polar auxin transport. Cellular vesicle transport depends on the distribution of actin filaments. Our results showed that actin filaments gathered into bundles under UV-B radiation. Which indicated such bundle structure affected the transport process of pectin-containing vesicles. All these observations restricted cell growth since the precondition of growth is the cell structure is normal.

The phytohormonal control of actin filament organization by auxin plays a key role in plant morphogenesis, which determines growth and differentiation of various plant organs (Blume et al., 2012). IAA suppressed growth of Arabidopsis roots not by affecting cell division but by reducing the length of the elongation zone. One possible reason is that IAA may have enhanced the formation of actin filaments bundles which inhibited cell elongation (Rahman et al., 2007) as shown in this paper. Recent studies have confirmed that UV-B radiation-induced root bending occurs through a flavonoid-mediated phototropic effect.
response to UV-B radiation in *Arabidopsis* (Wan et al., 2018). They also found that UV-B radiation increased the distribution of auxin on the nonradiated side of the root tips, promoting growth and causing root bending. Our results also showed that IAA content increased which may have led to the bending root formation in wheat. However, the ultimate reason for the bending root formation could be due to the remodeling of actin filaments.

As shown in Figure 6, wheat roots can sense UV-B radiation through an UVR8 dependent/independent pathway. This induced ROS burst and increased IAA content, which enhanced actin filament bundle formation and formation of the “bending root”.

5. Conclusion

Here we reported the response of wheat roots exposed to enhanced UV-B radiation. Our results showed that UV-B radiation decreased seedling growth and induced “bending root” formation. In the transition zone of the wheat root, ROS burst and the IAA content increased, which caused actin filaments to gather as bundles. These bundled actin filaments could be a key factor in cell expansion or mitosis. Together, these results confirmed that the reason enhanced UV-B radiation inhibited plant growth could be due to the altered distribution of actin filaments in plant cells.

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References


